BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME XXXV.

WOODS HOLE, MASS.

JULY TO DECEMBER, 1918

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS OF VOLUME XXXV.

No. 1. JULY, 1918.

GOLDFARB, A. J. *Effects of Aging upon Germ Cells and upon Early Development. Part III* 1

HANCE, ROBERT T. *Variations in Somatic Chromosomes* 33

YOUNG, R. T. *The Relation of Rhythms and Endomixis, their Periodicity and Synchronism in Paramecium aurelia* 38

COLTON, HAROLD S. *Self Fertilization in the Air-breathing Pond Snails* 48

PACKARD, CHARLES. *The Effect of Radium Radiations on the Development of Chætopterus* 50

No. 2. AUGUST, 1918.

COWDRY, N. H. *The Cytology of the Myxomycetes with Special Reference to Mitochondria* 71

SHINJI, GEORGE O. *A Contribution to the Physiology of Wing Development in Aphids* 95

FAUST, ERNEST CARROLL. *Eye-Spots in Digenea* 117

No. 3. SEPTEMBER, 1918.

Twentieth Annual Report of the Marine Biological Laboratory for the Year 1917 129

HOY, W. E., JR. *A Study of Somatic Chromosomes, II* 166

DUESBERG, J. *On the Interstitial Cells of the Testicle in Didelphys* 175

No. 4. OCTOBER, 1918.

RICHARDS, MILDRED HODGE. *Two New Eye Colors in the Third Chromosome of Drosophila melanogaster* 199

LANCEFIELD, D. E. *Scarlet, an Autosomal Eye Color Identical with Sex-linked Vermilion* 207

LUND, BARBARA LEE. *The Toxic Action of K C N and its Relation to the State of Nutrition and Age of the Cell as shown by Paramecium and Didinium* 211

KJERSKOG-AGERSBORG, H. P. *Bilateral Tendencies and Habits in the Twenty-rayed Starfish Pycnopodia helienthoides (Stimpson)* 232

No. 5. NOVEMBER, 1918.

GEORGE, W. C. *Experiments on the Determination of the Fate of the Gray Crescent Material in the Frog Egg* 255
HOSKINS, MARGARET MORRIS. *Further Experiments on the Effect of Heat on the Eggs of Cumingia* 260
BEHRE, ELLINOR HELENE. *An Experimental Study of Acclimation to Temperature in Planaria dorotocephala* 277

No. 6. DECEMBER, 1918.

BARTELMEZ, GEORGE W. *The Relation of the Embryo to the Principal Axis of Symmetry in the Bird's Egg* 319
PATTERSON, J. T. *Studies on the Biology of Paracopidosomopsis* . 362

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VOL. XXXV JULY, 1918 NO. 1

CONTENTS

		PAGE
GOLDFARB, A. J.	*Effects of Aging upon Germ Cells and upon Early Development. Part III.*	1
HANCE, ROBERT T.	*Variations in Somatic Chromosomes*	33
YOUNG, R. T.	*The Relation of Rhythms and Endomixis, their Periodicity and Synchronism in Paramecium aurelia*	38
COLTON, HAROLD S.	*Self Fertilization in the Air-breathing Pond Snails*	48
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PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

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All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXV. *July, 1918.* *No. 1.*

BIOLOGICAL BULLETIN

EFFECTS OF AGING UPON GERM CELLS AND UPON EARLY DEVELOPMENT.

PART III. CHANGES IN VERY AGED EGGS

A. J. GOLDFARB,

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In the previous two studies it was shown that, beginning with maturation, definite progressive morphologic and physiologic changes took place in the egg, both within and without the body, such as (1) an increase in volume of the egg, (2) a loss of surrounding jelly layer, (3) a retardation in the rate and change in the manner of forming the fertilization membrane, (4) a retardation and inhibition in cleavage. These changes served as measurable and corroborative evidences of the physiologic condition or vitality of the eggs of a female at any time, and served to measure the deterioration or loss of vitality with age.

There were other symptoms or evidences of physiologic deterioration, especially in late stages of ageing, or overripening, which will be discussed briefly in this paper. These changes include: (1) Agglutination of eggs, (2) fusion of eggs, (3) abnormal cleavage, (4) separation of blastomeres and (5) cytolysis of the eggs.

The same technique, the same nomenclature, the same precautions and the same three species of sea urchin were used as in Parts I. and II. For details of these I must refer to these studies.

AGGLUTINATION OF EGGS.

In Part II. it was shown that freshly removed eggs in good physiologic condition formed fertilization membranes within two minutes, at a rate accelerated with age, and with a wide peri-

vitelline space. With increasing age, or in freshly liberated eggs in poor physiologic condition, the rate of membrane formation was correspondingly retarded, and the perivitelline space was correspondingly diminished. In late stages of physiologic deterioration, the membrane-forming substance was entirely gone, and no membranes were formed. Synchronous with these changes in the fertilization membrane, other changes took place, of which the most important for my present purpose were, first a progressive loss of surrounding jelly layer of the eggs, and secondly a change in permeability of the cortical layer of the eggs which permitted an increasing volume of sea water to penetrate into the egg, increasing its volume and rendering the cytoplasm increasingly viscous.

When the eggs reached this stage of aging or physiologic deterioration, agglutination took place. Such aged or stale eggs had actually reached the condition that experimenters (Loeb, Driesch, Goldfarb and de Haan) have sought to produce experimentally.

Loeb used hypotonic sea water, which swelled the eggs and burst their membranes. Later he used hyperalkaline dilute sea water, which in addition made the cortical layer viscous. Driesch, Goldfarb, and de Haan removed the surrounding jelly and the fertilization membranes by mechanical treatment and then centrifuged the eggs in sea water or in hyperalkaline sea water. Goldfarb later used only such eggs as had been kept in the laboratory for about five hours, and without other treatment obtained much larger numbers of agglutinations. These investigators as well as Morgan, Nussbaum, Wilson, Zur Strassen and others had observed from time to time the spontaneous agglutination (and fusion) of eggs in different species of urchins, as well as in other animals. It is very probable indeed that in these instances spontaneous agglutinations occurred in the eggs that had sufficiently aged, and hence were in the condition most favorable for their ready agglutination. Certain it is that in all of my cultures of aging eggs, and in all three species, agglutination took place in every experiment in which the eggs were allowed to age over a sufficiently long period. The facts concerning the agglutination in aging eggs may be summarized as follows:

Agglutination sometimes occurred in freshly liberated eggs. But in every such instance when suitably tested, as described in Study I. and II., they were found to be in poor physiologic condition. Thus in experiment 1, agglutination was observed in one female, 9 minutes after the liberation of her eggs, in another, 29 minutes after liberation. But these eggs, in spite of their freshness, were in very poor physiologic condition.

Agglutination of the eggs of different females of an experiment often began at very different ages, depending upon the physiologic condition of the eggs when liberated. The more deteriorated the eggs, the earlier the agglutination. For example, the eggs of female 1, in experiment 1, first agglutinated in 9 minutes, female 3 in 29 minutes, female 2 in 225 minutes. These eggs were all in varying degrees of poor physiologic condition, those of female 4 which were in good physiologic condition did not agglutinate in the 309 minutes that the eggs were under observation. In experiment 3, the eggs of female 3 did not agglutinate until 8½ hours old, and the eggs of females 1 and 2 first agglutinated when six hours old. In experiment 4, the eggs of female 1 and 2 first agglutinated when 2½ hours old, female 5 and 6, when 8½ hours old; female 3 did not agglutinate in the 11 hours under observation, etc.

Once begun, agglutinations were observed throughout the remainder of the experiment. I am not certain to what extent there was, with increasing age, an increase in the number of agglutinated clusters, and to what extent there was an increase in the number of eggs to each cluster. Both kinds of increases took place in experiment 1, 2, 3, 4, etc. (*Toxopnuestes*), experiment 14 (*Hipponoë*) and experiment 17 (*Arbacia*), etc.

Agglutination occurred in fertilized as well as in unfertilized eggs, and in both groups each of the eggs in the cluster developed in a normal manner. The later regulations and the different types of larvæ resulting from such clusters I have elsewhere described in detail (Goldfarb, '13, '15). For the present I merely wish to emphasize the fact that agglutination took place long before the death and before extreme deterioration of the eggs.

Agglutination was independent of the age or physiologic condition of the sperm. Eggs in suitable condition agglutinated

whether fertilized or unfertilized. And when fertilized they agglutinated equally well whether old or fresh sperm were used, subject only to the following qualification, viz., when fresh sperm in high concentration were used, they caused the eggs to revolve rapidly in various directions, thereby preventing adjoining eggs from agglutinating, and separating many of the eggs already agglutinated. This was observed in *Toxopneustes*, experiment 1, 2, 3, 4; in *Arbacia*, experiment 16, 17; in *Hipponoë*, experiment 14, etc.

Clusters of 2 to 40 and more eggs were agglutinated to one another. Large clusters died in early cleavage. Clusters of 3 and 4 eggs often reached the blastula stage, and sometimes the gastrula stage, and then died. Clusters of 2 frequently reached the larva stage. The various types of gastrula and larvæ resulting from such agglutinated pairs of eggs and the processes involved, I have described in a previous publication (Goldfarb, '13, '15).

Agglutination occurred in every experiment in which the eggs had aged sufficiently, *i. e.*, in which physiologic deterioration had proceeded to a sufficient degree, as determined by the various tests enumerated in Study I. and II. Such deterioration gave rise without any other treatment, to exactly the conditions that were found to be most successful in the experimental agglutination of eggs, namely, loss of jelly layer, loss of membrane and a viscous condition of the naked protoplasm of the egg.

The rate of physiologic deterioration and hence the rate of agglutination depended upon the condition of the eggs at liberation, the species of egg used, the temperature of the sea water, and age.

Fusion of Eggs.

Some of the agglutinated eggs fused more or less completely together in all cultures of aging eggs. Only a small per cent. however fused, partly because clusters of more than 4 eggs died in early cleavage, while clusters of 3 or 4 eggs rarely survived beyond the blastula stage; and partly because clusters of 2 or 3 eggs often separated as a result of the uncoördinated ciliary activity of the different component blastula, gastrula or larvæ. Therefore only some of the closely agglutinated clusters of 2 or 3

eggs, fused together permanently, either in the egg stage, or in the course of subsequent early development. The processes involved in the fusion of eggs or embryos, and the various types of larvæ resulting from such fusions I have descibed elsewhere (Goldfarb, '13, '15).

For the present my interest lies not in the fusion process, per se, nor in the various correlated phenomena, but rather in fusion as another evidence or symptom of aging or deterioration of the eggs.

The observations may be summarized as follows:

"Spontaneous" fusion of eggs occurred in all three species of eggs, and in all cultures in which the eggs had aged sufficiently.

"Spontaneous" fusion occurred in unfertilized as well as in fertilized eggs.

In unfertilized eggs fusion was usually complete, giving rise to "giant" eggs which measured 8, 10 and 13 units diameter, whereas the control eggs measured but 6 units. These volumetric differences indicate, as Driesch pointed out, the number of eggs which were united into the single giant egg.

A number of observers have recorded the "spontaneous" occurrence of giant eggs or fused larvæ (Herbst, Morgan and Wilson, Nussbaum and Oxner). These spontaneously formed giant eggs or fused larvæ, it is very probable, arose as in my cultures by the natural deterioration of aging eggs, affecting the different structures of the egg in a manner so favorable to the fusion process.

Fusion occurred also among fertilized eggs. The resulting degree of completeness of fusion was conditioned largely, if not exclusively, by the stage in development when fusion first began. The earlier the fusion the more complete, and vice versa, the later, the less complete. Early fusion gave rise to "single" larvæ, either giant or normal. Late fusion gave rise to double larvæ of many types (Goldfarb, '15).

Fusion began at widely different ages, *i. e.*, at widely different periods after the liberation of the eggs. In *Toxopneustes*, experiment 6, the eggs of one female fused when 2 hours old, another when 5 hours old; in experiment 8, when 5 hours old; experiment 2, when 6 hours old; experiment 11, when 23 hours

old; experiment 9, when 25 hours old; experiment 12, when 50 hours old. In *Hipponoë*, the eggs fused when 23 hours old, and 42 hours in experiment 14. In *Arbacia* when 48 hours, experiment 17; 63 hours, experiment 20; 65 hours, experiment 19, etc. The explanation for this wide range in the onset of fusion within the same species, lies in the correspondingly wide diversity in the physiologic condition of the eggs of different females at the time of liberation, and hence correspondingly wide difference in the rate of deterioration.

Abnormal Cleavage.

Besides agglutination and fusion, there were other evidences of late aging, which might conveniently be grouped together under the term abnormal cleavage. Such abnormality included an increasing irregularity of shape and size of the blastomeres, an increasing lack of cohesion of the blastomeres, increasing retardation of mitosis, increasing numbers of atypic blastulæ, gastrulæ and larvæ, and an increasing inhibition of development.

These symptoms of extreme physiologic deterioration occurred essentially alike in all three species.

The onset of abnormality in cleavage occurred at widely varying ages, due to correspondingly wide variation in the physiologic condition of the eggs at liberation. For example, in experiment 4, the eggs of one female first showed marked irregular cleavage when 2⅔ hours old, another female when 5 hours old, others when 8½ hours old, etc.

After a long initial period, during which cleavage was regular, there was with further and extreme deterioration a progressive increase in the degree of irregularity and in the numbers of irregularly cleaving eggs. For example, in experiment 2, some of the eggs of females 2 and 3 became decidedly irregular, when 99 minutes old. From this time on, the number increased continuously until the eggs were 214 minutes old. Beyond this age, it was increasingly difficult to ascertain the ratio of irregular to the whole number of eggs because of increasing cytolysis and fragmentation of both unfertilized and fertilized eggs. In experiment 16, when the eggs were 1½ hours old, the average number of irregularly cleaving eggs of the 3 females was 7 per

cent. of the total. When 5 hours old, the number had increased to 46 per cent. of the total. In *Arbacia*, experiment 16, the average per cent. of irregularity of the 7 females when their eggs were 1½ hours old was 12 per cent. of the total. When 18½ hours old, 12 per cent. of the non-cytolized and non-fragmented eggs cleaved irregularly. When 24 hours old, the number of irregular eggs had increased to 40 per cent. of the non-cytolized eggs.

These deteriorated and irregularly cleaving eggs were short lived. With increasing age they died in increasing numbers, and at earlier and earlier stages in their development. *The more deteriorated the eggs, the less completely did they develop.* This differential mortality served as another index of the vitality or physiologic age of the eggs of any female.

These results give substantial support to Pearl's hypothesis of a varying degree of virility of the different eggs of an individual.

Irregular cleavage, with the consequences above enumerated, was due to several causes. In the first place the loss of jelly and the absence of the membrane made it increasingly possible for polyspermy to occur, particularly when fresh sperm in high concentration was used. Polyspermy in old eggs had been observed before by the Hertwigs, '86. These polyspermic eggs gave rise to irregular mitosis and irregular cleavage. In the second place the changed permeability, with its consequent excessive inflow of sea water, gave rise to mechanical or physical interference, dissolved certain of the protoplasmic granules, caused an excessive metabolism and an accumulation of metabolic products, gave rise to a very viscous condition of the protoplasm, etc., all of which were contributory agents in producing the various and increasing irregularities in cleavage.

Separation of Blastomeres.

Another quite characteristic manifestation of extreme age or physiologic deterioration was the more and more complete separation of the blastomeres. As the eggs increased in age, there was, as I have pointed out, an increasing viscosity of the cytoplasm and membrane, ultimately a complete loss of the fertilization membrane, or membrane-forming substance, with a

consequent enlargement and partial separation of the first two blastomeres, into an elongated double ball; and finally a more and more complete separation. The blastomeres fell apart completely.

There can be no doubt that F. R. Lillie ('14) and Loeb's observations that blastomeres do fall apart in old eggs is correct.

Separation of blastomeres occurred in every experiment in which the observations were made over a sufficiently long period. And once begun the blastomeres continued to separate with successive cleavages until there was in place of the egg, a flattened mound of minute blastomeres, stuck to the bottom of the dish or to one another. And with increasing age, more and more eggs passed through this history.

As might have been anticipated, the onset of separation varied with different females according to the physiologic condition of the eggs at liberation. Separation was first observed in experiment 3, when the eggs were 6 hours old; in experiment 4, when 5 hours old (1 female) and 8 hours old (in the other 2 females); in experiment 5, 8 hours old in one female, later, in the other 5 females; in *Arbacia*, the eggs of one female, experiment 16, showed the separation phenomenon when 18 hours old, 2 other females when 24 hours old, and 2 others when 42 hours old. In experiment 15, separation was observed in 3 females when eggs were 24 hours old, in experiment 17 when 23 and 28 hours old, etc. In *Toxopneustes*, and *Hipponoë*," separation usually occurred in 8 to 10 hours after liberation, in *Arbacia* usually not before 24 hours.

With the continued and complete separation of the blastomeres in successive cleavages, an increasing number of the fertilized eggs died and with their disintegration many of the non-separating eggs also died.

There can no longer be any doubt that there were no fertilization membranes about these aging eggs. Glazer ('14) urged that separation was due to the absence of the fertilization membrane. It is far more probable that the absence of the membrane together with the physiologic changes in the protoplasm (the result of aging) are both responsible for the separation of the blastomeres.

Cytolysis of Ageing Eggs.

The consummation of the various deteriorating changes in aging eggs is cytolysis and death.

Cytolysis of sea-urchin eggs under the influence of various experimental conditions, such as saponin, salycyl aldehydes. propyl alcohol, distilled water, etc., have been carefully described by Loeb. Loeb speaks of two methods of cytolysis, which he calls "white" and "black."

Aging eggs cytolize in the same two ways. In the "white" or cytolysis by liquefaction, a changed permeability of the cortical layer permits an increasing volume of sea water to enter the egg with a corresponding enlargement of the egg, a more viscous condition of the cytoplasm, a diminution in size of the protoplasmic granules, and an increasingly hyaline and translucent appearance of the egg. In the second type of cytolysis, there is either far less increase in size of the egg or no increase at all; the central mass remains opaque and becomes increasingly opaque; the cytoplasm is far less viscous; the outer surface which is hyaline is fragmented, and sometimes the inner mass as well, is fragmented, and the outer fragments fall off, with a consequent diminution in size of the eggs, even far below the norm.

I was unable satisfactorily to establish whether these two are independent methods of cytolysis, possibly associated with different degrees of virility of the eggs or whether they are sequential phenomena. In most cultures, both types of cytolysis are seen at the same time.

The onset differs in the eggs of different females, and, as in other evidences of aging, this variation is due to differences in the physiologic condition of the eggs at liberation. Those eggs which were in good physiologic condition at liberation, cytolized late; those in poor condition, early. Eggs in relatively similar physiologic condition cytolized at a similar rate at the same temperature. The greater the temperature, the greater the rate.

In *Toxopneustes*, cytolysis in any considerable numbers, was first observed when $\frac{1}{5}$ hour old in experiment 1, $\frac{1}{5}$ hour old in experiment 3, 6 hours old in experiment 2 and 5, 11 hours old in experiment 4, 20 hours old in experiment 9. In *Hipponoë*, the rate of cytolysis is essentially the same as in *Toxopneustes*.

In *Arbacia* it was much slower. Beginning, in the given experimental conditions, in about 28 hours as in experiment 17, and extending to 42 hours as in series 19, 46 hours in experiment 18, and later in other experiments. This difference in rate of cytolysis is in part due to differences in temperature of the sea water in the two localities, but it is also due, and is another evidence of, a protoplasmic difference in the two species of eggs.

Correlation of the Changes in Ageing Eggs.

I have for the sake of clearer exposition described each type of change in aging eggs, such as agglutination, irregular cleavage, etc., as though each type of change was independent and unrelated to the other changes. *I wish now to emphasize the entirely dependent, related and correlated character of the changes I have heretofore treated separately.*

Seven types of changes and tests have been enumerated, whereby the physiologic condition of the eggs may be accurately determined. These tests are (1) size of the egg, (2) per cent. possessing jelly layer, (3) rate and width of membrane, (4) rate, total and character of cleavage, (5) rate and degree of agglutination, (6) rate and degree of fusion of eggs, (7) and per cent. of cytolysis. *Each test serves as a verifiable measure of qualitative and quantitative changes in the vitality or physiologic condition of the eggs under the given conditions.*

But more significant for my present purpose is the fact that the variation in any one of these seven tests is associated with corresponding variation in the others. *So highly correlated are they that a knowledge of one enables one to predict with a remarkable degree of exactness, the degree of variation in the others.* If the cleavage rate for example, is known, one could predict the size of the eggs, the per cent. with jelly layers, the rate of fertilization membrane, the per cent. of agglutination, etc. This somewhat sweeping statement is made with the understanding that it is subject to the qualifications referred to in the text.

It is sometimes inconvenient or difficult to take certain precautions with respect to one or another of the seven groups of tests, and without such precautions any one test may give a large error. But the ensemble of the tests or any considerable

group of them rectifies any such error, and gives an accurate measure of the physiologic condition of the eggs. The *determination by several or all of the tests serves as the most exact method so far recorded, whereby the vitality of any batch of eggs may be accurately ascertained and whereby the rate and nature of the manifold changes, with age or under any set of experimental conditions, may be determined.*

The correlated changes are given below in tabular form.

	Physiologically Good Eggs. "Fresh" Eggs.	Physiologically Poor Eggs. "Aged" Eggs.	Physiologically Bad Eggs. "Very Aged" Eggs.
Size:	Small deviation from norm.	Increasing deviation. Increasing size.	Further deviation. Further increase above or decrease below norm.
Jelly:	All or nearly all eggs with jelly	Increasing per cent. without layer.	Further per cent. without layer.
Membrane formation:	(*a*) Formed within 2 minutes.	Increasingly retarded.	None.
	(*b*) Wide space between eggs and membrane.	Increasingly narrow.	None.
Cleavage:	(*a*) Maximum per cent. of eggs cleave.	Increasingly lower per cent.	Further reduction.
	(*b*) Maximum rate of cleavage.	Increasingly retarded.	Further retardation.
	(*c*) Minimum irregular cleavage.	Increasing per cent. irregular.	Further irregular.
	(*d*) No separation of blastomeres.	Increasing per cent. separate.	Further separation.
Agglutination:	None of the eggs agglutinate.	Increasing number agglutinated.	Further agglutination.
Fusion:	None of the eggs fuse.	Increasing number fuse.	Further fusion.
Cytolysis:	None of the eggs cytolized.	Increasing number cytolyze.	Further cytolysis.

This remarkably close correlation of the various changes associated with aging may be expressed graphically as in Fig. 1. The ordinates represent the degree of change or physiologic deterioration, the abscissas represent age of eggs. There are a number of independent tests which demonstrate unequivocally that freshly liberated eggs (in good physiologic condition) improve with age, and after a definite period begin to deteriorate. Such eggs upon liberation produce their membranes increasingly fast, and cleave more rapidly, and with increasingly large per cents, as the eggs become older. This gradual improvement or

super-ripening is represented in the heavy lined curve (Fig. 1).[1] The process continues until an optimum is finally reached many hours after liberation at a stage marked *B*. Beyond this age there occurred a definite progressive deterioration, indicated by

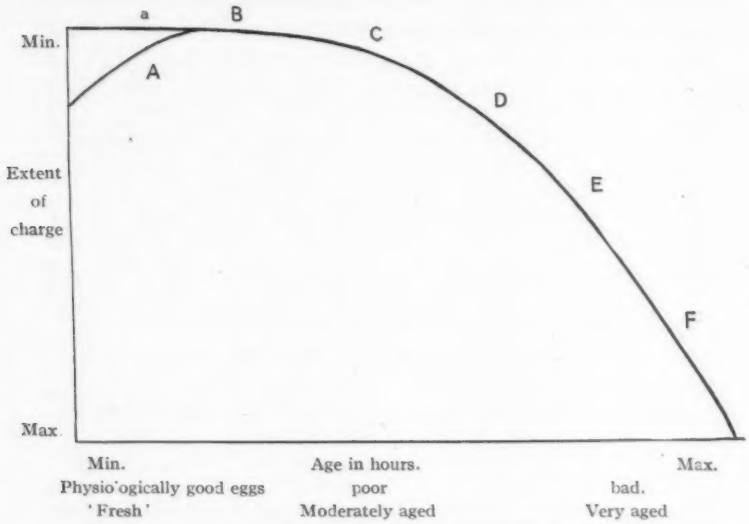

FIG. 1. Showing extent of morphologic and physiologic changes during ageing or deterioration of eggs. *AB* represents the period of superripening; *BCD*, the period of overripening; *DEF*, the period of extreme deterioration and death. *ABCDE* represents change in volume, rate of membrane formation, rate of cleavage, total cleavage (*Hipponoë*). *aBCDE* represents loss of jelly, total cleavage (*Arbacia*), width of membrane formation. *CDEF* represents increase in agglutination, fusion, abnormal cleavage, separation of blastomeres, cytolysis, and increase in oxidation.

the progressive retardation in the rate of membrane formation, in the rate of cleavage, in the decrease in total cleavage, etc. With further aging, there appeared other symptoms that marked further deterioration, such as agglutination, fusion, irregular cleavage, separation of blastomeres, and finally cytolysis and death. Some of these symptoms first appeared at *C* of the curve, such as agglutination, others at *D* such as irregular cleavage, etc.

[1] *I. e.*, part A of the curve.

Discussion.

A number of investigators have been struck by the unexpectedly large variability in the eggs of different females of sea urchins, even when the eggs were liberated at the same time, and kept under apparently identical conditions. A few examples and the explanations offered to account for this diversity may be cited.

Stockard observed "that a number of eggs when subjected to the same solutions do not all respond in a like manner," and held that such variability was due to "differences in individual resistance and vigor."

F. R. Lillie also noted "the failure to obtain exactly the same curve . . . ," and believed the result "was due in part to the natural variability of different lots of eggs and sperm."

Loeb, J., and Wasteneys, H., also believed that this variability was due in large part to differences in the eggs.

Wasteneys later came to the conclusion that this variability was due to differences in sensitiveness of eggs of different females, and perhaps to temperature.

R. S. Lillie also held that variability was due to the condition of the eggs.

F. R. Lillie came closer to an understanding of the matter when he pointed out that "the condition of the eggs whether ripe or immature, fresh or stale, with or without jelly, is more important than concentration of the eggs. The condition of the gonad" he adds, "is the most variable thing in summer sea urchins."

Loeb ('02) came still closer to the truth, when he stated that "this (variability in maturation of asterias eggs) probably depends on the fact that the eggs of different females are not all in the same condition of ripeness."

Many other observations might be mentioned in which the authors have either ignored this large variability or, having noted it, ascribed the result to individual resistance, sensitiveness, natural variability and other equally indefinite causes.

In this connection one must mention Loeb and Chamberlain's work. These authors distinguished between the primary variation in the eggs of a given female, from the secondary variation

in the eggs of different females. They ascribed the primary variation to a difference in the amount of enzyme present in the different eggs of the same parentage.

In earlier studies, I showed that the ripe eggs from different freshly collected females varied far beyond Loeb's individual or primary variation. I determined *the exact range of variability of the freshly removed eggs, not merely in one but in several morphologic and physiologic entities, and for three species of sea urchin. I showed moreover that these variations were measurable and corroborative indices of differences in the vitality or physiologic condition of the different lots of eggs. And hence it was possible not only to ascertain with exactitude the condition of the eggs but to sort them out according to their physiologic condition.*

This unusual diversity in freshly liberated eggs of different females is due, firstly to the original or primary variability of Loeb and Chamberlain already referred to; secondly, to injurious changes that take place within the body of the female before liberation, and even as early as the maturation of the egg. For as soon as maturation is completed the eggs are susceptible to injurious agencies in the body fluid of the mother, particularly changes in ionic concentration and oxygen content. Whether these be the sole injurious agents or whether accumulated toxic (metabolic) substances also contribute, I cannot say. In any event, the equilibrium is upset and the *cycle of physiologic deteriorative changes which begins with maturation, proceeds at varying rates depending upon the length of time the eggs are held within the body, and the nature of the body fluids.*

Since the eggs of different females may mature at different times, and the eggs of a given female mature at different intervals, and since the body fluids either cause or permit deleterious changes to take place, and finally since the time between maturation and liberation may vary very widely in different females, it is not surprising then that such marked differences obtained in the different lots of eggs. These marked variations occurred in the two southern species probably throughout the breeding season; in the northern species the variation was less marked during the height of the breeding season.

Hence to assume that ripe eggs of different females, or that

all the eggs of a given female are in the same or nearly the same physiologic condition, when freshly liberated, is misleading and frequently very inaccurate.

It has been held by Minot that senescence of the individual began, not after sexual maturity, but far back in the history of the individual, even before birth. My studies on aging eggs lead me to the conclusion that senescence of the individual may be traced as far back in the history of the individual as the maturation of the egg. The aging eggs show numerous evidences of senescence, not only during the egg stage but throughout their development including their larval stage. And it is very probable indeed that the effects are carried into the adult stage of the individual.

The value of the results depends upon the acceptance of the technique used. The freshly liberated eggs were placed under conditions that preliminary tests had shown were optimum for that particular species of sea-urchin egg, namely: (1) the sea water used had been collected at the height of the incoming tide, and so obtained a more constant grade of sea water, and more free of detritus, etc., (2) the sea water was filtered (with certain precautions) and stored in glass; (3) standard flat bottom bowls were used, in which the concentration of eggs was far below the injurious limit, and nearly constant for the different series; (4) the volume of sea water was large enough to prevent interference by evaporation, etc. (250 c.c.); (5) the supernatant sea water as well as the bowls were changed at least once a day; (6) the temperature was kept fairly constant and close to that of sea water in the open, etc.

Under these conditions the freshly liberated eggs (whatever their physiologic condition may have been at the time of liberation) underwent a graded series of morphologic and physiologic changes, which were not new processes or changes, but the continuation of the same changes which took place within the body before liberation, namely, loss of jelly, retardation of membrane formation, increase in size of the egg, decreased rate of division and decreased ability to cleave, etc.[1]

The rate of change was however accelerated. Such accelera-

[1] And it is very probable indeed, that these changes occur in a state of nature.

tion depended upon three factors, namely, the condition of the eggs when liberated, the time after liberation (age), the temperature. The more deteriorated the eggs at liberation, the greater the rate of change at any given interval thereafter. Likewise the longer the period (age) after liberation, the greater the rate of change or senescence. And finally the higher the temperature of the sea water, the faster the deterioration, closely approximating the expectation from Van't Hoff's law. The sea water at the Tortugas was approximately 10° C. higher than at Woods Hole, and the rate of deterioration was more than twice as great in the former as in the latter.

With further aging and increasing rate of change, other symptoms of deterioration became manifest and were increasingly intensified, such as agglutination, fusion, abnormal cleavage and cytolysis. These have been described and measured for the three species.

If the germ cells of other tropical organisms behave as those of the two tropical species of sea urchins, *i. e.*, die or become infertile, or are unable to develop beyond an early stage, even a few hours after liberation, one may account, at least in part, for the relatively few individuals that reach maturity in so many tropical species.

The eggs that do survive show profound changes in nearly all and possibly all the parts of the egg. A full appreciation of this fact enables one to correlate and to find a common explanation in apparently unrelated experiments.

A number of investigators have noted a decreased productivity with increasing age of the mother. Minot ('91) came to this conclusion from his experiments with guinea pigs. Marshall's observations upon women, Pearl's upon fowls and lambs, Hammond's upon rabbits and pigs, and recently King's upon rats, all led to the same conclusion. Two explanations appear to me to account for the results, both of them involving the deterioration of the egg. Either the gonads of old mothers produce eggs in lower physiologic condition than younger mothers, or the eggs are of the same or similar physiologic condition at any age, but the interval between maturation and fertilization is greater in older mothers and hence the eggs in poorer physiologic condition

when fertilized. In either event the end result seems to be essentially the same as in the sea-urchin eggs, namely fewer develop, more become irregular or abnormal, with the age of the mother.[1] The early history of the changes in the sea-urchin egg and sperm as they deteriorate, we now know, and it is very probable that the eggs of aging mothers undergo fundamentally similar changes.

The experiments with aging eggs throw light upon, and indicate the probable cause of seemingly conflicting results in hybridization experiments.

The Hertwigs ('86) affirmed in contradistinction to the conclusions of Pfluger ('82) and Born ('83) that hybridization took place the more readily when the eggs were of lower vitality. Vernon ('98) also noted that the chances of hybridization were increased when the eggs had previously been kept for about nine hours in sea water, which, I have shown, lowers the vitality of the eggs. Hagedorn ('09) discovered another method for increasing the chances of hybridization, namely by increasing the HO concentration of the sea water. Herbst, Tennent, Godlewsky-Loeb and Kupelweiser used various modifications of Hagedoorn's method and obtained similar results. But as will be later shown, the increased alkalinity of the sea water only served to increase the rate of deterioration of the eggs, and hence prematurely aged them; these eggs showed the same changes which I have described in aging eggs, changes which permit the ready entrance of multiple autogenous sperm, or heterogenous sperm, and hence give rise to abnormal development or to hybridization.

Similarly, Doncaster, Vernon, and Herbst observed that hybridization could be increased by raising the temperature of the sea water. This also I have shown serves merely to precociously age the eggs and hence makes greater hybridization possible.

Less effective was the use of dilute sea water used by Tennent and Herbst. Here again, dilute sea water increased ionization, and hence increased the metabolism of the egg, which in turn increased the rate of aging.

[1] The age of the sperm or the father is far less important than the age of the egg or of the mother.

All these apparently diverse methods have this in common, viz., that they all induce the same changes in the eggs, i. e. accelerate the aging of eggs, and thus permit the ready entrance of autogenous or heterogenous sperm. Instead of emphasizing the one or the other external agent or change, as Herbst has done, I prefer to emphasize that which is common to all, namely, the very definite morphologic and physiologic changes within the egg, which I have termed physiologic deterioration or aging.

There are on record other phenomena which may now be interpreted in terms of the known changes in aging eggs, and which may be briefly summarized as follows:

Vernon ('95, '98) observed a seasonal change in the size of the larvæ, in the per cent. of hybridization, in the per cent. reaching the larval stage, in the maternal or paternal type of hybridized larvæ. These seasonal changes are in all probability due, as he himself suggested, to the varying physiologic conditions of the eggs, at different periods of the breeding season.

Fuchs ('14) observed that the per cent. of self-fertilizations in *Cione* increased with the age of the eggs. This is exactly what one should expect from the known changes in aging eggs and of the ready entrance of sperm in old eggs.

Matthews ('01) could induce parthenogenesis by shaking (in *Asterias forbesii*), most readily, when the eggs were at least three hours old.

De Vries, according to Vernon, obtained a larger per cent. (40) of mutants in old seeds, whereas fresh seeds gave only 1 to 5 per cent. This suggested that not only may the protoplasmic parts of the egg be radically modified by aging, but that the nuclear parts may be correspondingly affected.

Other experiments serve to strengthen this conclusion, particularly those upon sex determination.

R. Hertwig ('06 and '07) and Kuschekewitch were first to note that sex could be altered by aging the eggs of the frog. More recently Riddle ('12–'14), experimenting with pigeons, concluded that the sex of the offspring was conditioned by the physiologic activity of the reproductive organs or by the age of the mother. Both hyperactivity and aging of the mother causes an excess of male-producing eggs.

Stout has reported similar results in *Cichorium*.

King experimented with toad eggs and succeeded by drying the eggs in materially changing the sex ratio.

If it should be established that in these instances a change in sex was really induced by the experimental condition, it could be readily shown that in all these instances a physiologic deterioration of the eggs was the primary cause and affected profoundly not merely the cytoplasm but the nuclear substance of the eggs as well. It may be possible that aging modifies or destroys the chromosomes in the same way that particles of the cytoplasm are modified or destroyed.

For, recent studies of Marine and Manley, of Patten, of MacNider and others point unmistakably to the conclusion that in aging adult individuals there occur definite changes in different tissues of the body, as well as in the blood stream, changes which are profound in extent and correspond very closely to those I have described in aging eggs. In view of these observations I am led to believe that the reproductive tissues and the eggs of older mothers would be correspondingly modified. And it is also probable that the cyclical change in the aging eggs correspond with and are caused by corresponding cyclical changes in the parental tissues. For it will be recalled that the eggs showed an initial period of increasing ripening, increasing fertilizability, increasing rate of development, etc., until an optimum condition was attained, and then there followed a long period of progressive deterioration, modification and ultimate disintegration.

Returning now to the consideration of the changes in aging eggs, the question at once suggested itself. What then is the fundamental nature of the physiologic deterioration, which began with maturation and ended in the complete cytolysis of the egg. The changes are twofold. There is, on the one hand, a dissolving of the surrounding jelly layer, and, on the other hand, a gelatinization and dissolution of the membrane-forming substances in the cortical layer of the egg. These twofold changes are parallel. I find myself at variance with Harvey who held that the loss of jelly under "normal" conditions was independent of aging of the egg. My observations clearly show that if proper precautions against mechanical shaking and changed HO

ion concentration be made, the loss of jelly is concomitant with physiologic deterioration or aging of the eggs.

The second and more important change is concerned with the cortical layer of the egg. With age the dissolution of membrane-forming substances progresses and the permeability of the cortical layer increases, as a consequence of which a number of other changes occur, namely: (1) An inflow of sea water and an increase in size of the egg; (2) increasing mechanical interference due to the excess sea water; (3) thinning and final disappearance of fertilization membrane; (4) gradual solution of protoplasmic granules; (5) increasing viscosity of the cytoplasm; (6) increasing metabolism and metabolic products.

By aging is meant the ensemble of these and other changes in the egg.

It is not to be wondered then that with protecting jelly, cortical layer, cytoplasm and probably nucleus as well, chemically and physically altered, profoundly altered in some instances and entirely gone in others, that the subsequent history of the egg should show evidences of these changes, and be correspondingly altered in development. These changes in development may be categorically summarized as follows: (1) Increasing retardation and final loss in membrane formation; (2) increasing modification in character of membrane; (3) increasing retardation and final cessation of cleavage; (4) increasing irregularity in cleavage; (5) increasing viscosity of cytoplasm and cortical layer; (6) increasing agglutination and fusion of eggs; (7) increasing separation of the blastomeres; (8) increasing modified larvæ, etc.

The problem of physiologic deterioration or "aging" is reducable then to two main factors (1) a change in permeability of the cortical layer, (2) a change in the metabolism of the egg. I have already outlined the nature and conseqences of the first of these two factors. A few words may be added concerning the second.

Loeb's researches on oxidation of eggs had led him to the conclusion that with "aging," the eggs had increasing difficulty in disposing of the accumulated store of toxic metabolic products. Wasteneys subsequently by direct experimentation showed that there was a definite increase in metabolism with aging of eggs.

My own observations, still unpublished, extend and corroborate Wasteneys's results.

But to what extent was the change in permeability of the cortical layer, with its train of events, responsible for the deterioration of the eggs. If it was an important factor, it should be possible experimentally to retard these cortical changes, and hence correspondingly to increase the longevity and to retard the deterioration of the eggs. Such experiments were made repeatedly and successfully, an account of which will appear later. I mention these experiments merely to emphasize the fact that there appears at present two methods by which physiologic deterioration and "aging" may be controlled, namely, by retarding or preventing either the metabolism, or the change in permeability of the eggs.

By reducing the metabolism of the eggs, Lyon, Loeb and Wasteneys retarded the deterioration of the eggs. By retarding the cortical changes I have retarded the deterioration of eggs as well. By a summation of both methods far greater control of aging or deterioration was obtained.

Both methods are closely related. A change in the cortical layer induced a corresponding change in the metabolism of the egg, and it is probable that the reverse obtains, namely a change in metabolism is associated with a change in the cortical layer.

This leads directly to the possibility urged by Loeb, Child and, particularly in view of the unpublished work of E. J. Cohen,[1] that ageing and hence longevity of the germ cells is a function of the rate of metabolic activity of the protoplasm of the egg or sperm, the greater the rate of metabolic activity, the shorter the life of the germ cell, and vice versa, the lower the rate of metabolism the longer lived the germ cell.

Hence eggs in sea water are longer lived than dilute concentrations of sperm, both of the same chronologic and physiologic age. For the same reason eggs in sea water are shorter lived than concentrated or dry sperm. For in this condition the sperm are inactive, and hence lower metabolic rate. How far the result is due to the greater metabolism on the one hand as

[1] The first study has since appeared in the BIOLOGICAL BULLETIN, Vol. 34, 3, 1918.

urged by Child, or to the greater accumulation of metabolic toxic products as urged by Loeb, or to both of these factors, we cannot in our present state of knowledge determine.

Summary.

Aging eggs show progressive measurable morphologic and physiologic changes. Besides those described in Study II., there are a number particularly evident in later stages of aging eggs, such as agglutination and fusion of eggs, irregular cleavage, separation of the blastomeres and cytolysis.

A. 1. The agglutination phenomenon occurred only in physiologically very deteriorated or "aged" eggs, as evidenced in part by the low per cent. of the jelly layer, inability to develop a fertilization membrane, a more viscous condition of the cytoplasm and cortical layer, and by numerous other tests. These are exactly the conditions that have been sought in the experimental agglutination of eggs.

2. The onset of agglutination occurred at different ages for the eggs of different females, such variations being due to differences in physiologic condition of the eggs at the time of liberation from the parent. The more deteriorated the eggs at liberation the earlier the agglutination, and vice versa. Agglutination occurred in every experiment in which the eggs had aged sufficiently.

3. Some eggs remained agglutinated throughout their subsequent developmental history, others were secondarily separated at varying swimming stages in development, others died precociously, due to asphyxiation.

4. Fertilized as well as unfertilized eggs were agglutinated.

5. Clusters of 2 to 40 or more eggs were thus agglutinated.

6. Agglutination is not determined by the condition of the sperm except in so far as concentrated fresh sperm may, by revolving the eggs, separate agglutinated eggs or prevent their so doing.

B. 1. When the eggs were in sufficiently poor physiological condition, as determined by suitable tests, they tended not merely to agglutinate but many subsequently fused more or less completely.

2. Such fusion may occur in the egg stage or during subsequent development, with corresponding complete or incomplete fusion. The degree of fusion determined the various types of fused embryos and larvæ described in previous publications.

3. Complete spontaneous fusion of not more than three eggs occurred not infrequently and gave rise to giant eggs.

4. Fusion occurred in all experiments in which the eggs were allowed to age sufficiently.

5. The variation in the time of fusion was determined by the physiologic condition of the eggs at liberation. The more deteriorated the sooner the fusion.

6. Fused eggs and embryos, and giant eggs occurred in all three species of sea-urchin eggs, namely, *Arbacia*, *Hipponoë* and *Toxopneustes*.

7. It is probable that the "spontaneous" fusions of larvæ and embryos, described by many workers, find their explanation in similar aging or deterioration of the eggs.

C. 1. With increasing age and physiologic deterioration of the eggs, cleavage was increasingly irregular. This irregularity was manifested in a change in size and shape of the blastomeres, in retardation in the rate of cleavage, in increasing inhibition at progressively earlier stages of development, increasing numbers and types of atypic embryos and larvæ, and in extreme stages, in total lack of cleavage.

2. In every batch of eggs irregular cleavage occurred as soon as the eggs had aged sufficiently. The onset and the degree of irregularity varied with the physiologic condition of the eggs when liberated. Once begun there was a progressive increase in the numbers and in the degree of abnormality.

3. Irregular cleavage which is a consequence of aging is due in part to excessive intake of sea water, and in part to polyspermy. The quantitative relations of these two factors was not determined. Both are due to a change in the cortical layer of the egg.

4. Deteriorated or irregularly cleaving eggs, were shorter lived than physiologically fresh eggs. The greater the irregularity (or physiologic age) the short lived the eggs, and vice versa, the more virile the egg the greater the longevity and the less irregular development.

D. 1. With increasing physiologic deterioration, there occurred another type of irregularity, namely, more or less complete separation of the blastomeres.

2. Such separation occurred in every experiment that was carried over a sufficiently long period, and with further aging separation of blastomeres was increasingly complete.

3. The separation of the blastomeres occurred with successive cleavages.

4. In *Toxopneustes* and *Hipponoë*, separation usually occurred eight to ten hours after physiologically good eggs were removed from the female. In *Arbacia* about 24 hours were required. The variation in onset in different females was due to variation in the physiologic condition of the eggs at liberation.

E. 1. With still further physiologic deterioration (or "ageing") cytolysis set in. There were two types of cytolysis, namely cytolysis by liquefication or enlargement, and by fragmentation or reduction.

2. The onset of cytolysis differs in the eggs of different females depending upon the physiologic condition of the eggs at the time of liberation.

3. Cytolysis occurred three or more times as rapidly in *Toxopneustes* and *Hipponoë* than in *Arbacia*. This difference is in part due to accelerated metabolic rate at the higher temperature of the southern species, partly due to increased HO ion concentration of sea water at Tortugas and partly to protoplasmic differences of the different species of eggs.

F. Agglutination, fusion, abnormal cleavage, separation of blastomeres and cytolysis of the eggs are phenomena correlated with intense physiologic deterioration of the eggs, and were observed in all cultures in which the eggs were sufficiently deteriorated.

Seven independent groups of tests were used to determine the degree of deterioration. These tests corroborate one another. Any one test, with suitable precautions, measures the vitality of the eggs. From any one, the other manifestations of aging may be predicted. A group of tests offers the most convincing means of measuring exactly the degree of senescence of any sample of of eggs.

These seven groups of tests or symptoms of senescence also indicate the fundamental nature of the chemical and physical changes involved in the aging process, namely a change in the cortical layer of the egg, and a change in oxidation. The change in the cortical layer, *i. e.*, the change in permeability, affects the membrane, the cleavage, and all the other consequences of aging above enumerated.

It is very probable that the changed cortical layer with its train of consequences, as well as the change in respiration, are fundamentally reducible to the one phenomenon, namely, changed metabolism.

These results afford a common explanation of apparently diverse phenomena, such as change of sex with age (Riddle, Hertwig, etc.), senescence (Minot), reduced productivity (Pearl, King, etc.), physiologic differences in cross fertilizations (Tennant, etc.), etc. (For details see discussion.)

TABLE I.

SHOWS VARIATION IN ONSET AND INCREASE IN AGGLUTINATION, FUSION, IRREGULAR CLEAVAGE, AND CYTOLYSIS WITH DETERIORATION AND AGE. A STANDS FOR AGGLUTINATION; F, FOR FUSION; S, FOR SEPARATION OF BLASTOMERES; I, FOR IRREGULAR OR ABNORMAL CLEAVAGE; C, FOR CYTOLYSIS; N, FOR NORMAL.

Exp. No.	Date.	Age of Germ Cells in Minutes.		Female Number.						Remarks.
		♀.	♂.	1.	2.	3.	4.	5.	6.	
1	7/2	9	9	A	N	C I	N			*Toxopneusles*
		29	29	A I	N	A C I	I			
		64	64	A I	N	A C I	C I			
		209	19	A I	C I	A C I	C I			
		225	35	A I	A C I	A I	I			
		250	60	A I	A C I	A I	I			
		309	120	A I	A C I	A I	I			
2	7/12	17	17	N	N	N				
		42	42	N	N	N				
		99	99	N	I	I				
		161	161	N	I	I				
		214	214	N	I	I				
		294	294	N	I	A I				
		360	360	A C	A I	A F I				
		420	420	A C	A I	A F I				
		436	60	I	I	I				
3	7/14	20	20	N	N	N				
		80	80	N	C	N				
		140	140	N	C	N				
		210	210	N	C	N				
		280	280	N	C	N				
		370	370	A S	A S	S I				
		440	440	A S	A S	S I				
		500	500	A S	A S	A S I				
		600	2	S I	S I	S I				
4	7/16	160	160	A I	A I	N	N	N	N	
		300	300	A I	A I	S I	N	N	I	
		500	500	A S I	A I	S I	I	A I	A I	
		670	19	S I	C I	S I	I	I	I	

* means many; *v* means very many; *f* means few individual specimens were agglutinated or cytolized, etc.

TABLE I.—*Continued.*

Exp. No.	Date.	Age of Germ Cells in Minutes.		Female Number.						Remarks.
		♀.	♂.	1.	2.	3.	4.	5.	6.	
5	7/19	130	130	N	N	N	N	N	N	
		240	240	N	N	N	N	N	N	
		350	350	N	N	N	C I	C	I	
							*			
		470	470	S	C I	C I	C I	C	C I	
		580	15	S	C I	C I	C I	I		
6	7/21	120	120	I	A F					
		330	330	F I	A					
7	7/7	75	75	N	N	N	N			
		110	110	N	N	N	N			
		470	10	S	N	N	N			
8	7/5	300	120	A S F I						
				* *	* *					
9	7/4	1200	20	A C I	A C I					Cooled 10° F.
				* * *	v * *					
		1200	20	A C I	A C I					75° F.
				*	*					
		1380	103	C	C I					
				*	*					
		1400	230	C	C I					
				*	*					
		2750	52	C	C					
		1500	20				F			
10	7/7	2790	20	S C	S C	S C	S C			
				*	v	v	*			
11	7/8	1390	21	S F I	S C I	S I	S I			
				*	*	*	*			
12	7/9	3040	21	F	F	F	F			
		hrs.	hrs.							*Hippo-*
13	6/19	23	23	S F I						*noë.*
		18	24	S						
14	6/24	24	12	F I						
				*						
		42	29	A S I						
				*						
		42	0	A S I						
				*						
		29	29	A S						
				*						
		29	0	A S						
15	8/14	1½	1½	I	I	I	I	I	N	*Arbacia.*
		5	5	I	I	I	I	I	N	
							*			
		24	1	S I	S I	I	I	S I	A I	
				*						
		49	1¼	A S I	A S I	A S I	A S I	A S I	A S I	

TABLE I.—*Continued.*

Exp. No.	Date.	Age of Germ Cells in Hours. ♀.	♂.	Female Number. 1.	2.	3.	4.	5.	6.	Remarks.
16	8/16	1½	1½	I	I	I		I	I	
		18	18	I	I	I		I	S I	
		23	23	A I	A I	I		A F I	A S	
		24	4	I	S I	I		S I	S I	
		42	1	S I	S I	S I		A F C S I	A F C S I	
		42	1/6	S I	S C	S C I		A F C S	C	
		48	1/6	S I	S I	S I		A F C S		
		65	¾	F S I	S I	S		A F		
		70	1/11	S I	S I	F				
17	8/13	23	23	N	S	A	A			
		28	4	A S I	A S I	A S I	A	S I		
		28	28	A C	A C	A C	A	C		
		48	½	A F	A F C	A	A	A		
		49	½	A I	A	A	A	A		
	8/13	4	4½	I	I	I	I	I		
18	8/12	48	48	I						
		48	48	I						
		18	48	I						
		18	18	I						
		1/16	48	*f* I						
		1/16	18	*f* I						
19	8/18	1	23	N	N					
		1	45	N	N					
		1	68	N	N					
		5	73	N	N					
	8/18	42	1/6	S I	C	C I	A F C I	A F C I	C I	
		48	1/6	S I	C S I	S C I	S C I	C I	C I	
		65	¾	F S I	C S I	S C I	S C I	C I	C I	
		70	1/11	F S I	C S I	F S C I				
	8/18	7	25	I S	S I	S I				

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VARIATIONS IN SOMATIC CHROMOSOMES.

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CONTENTS.

Introduction 33
The Cytological Conditions in *Œnothera scintillans* 34
The Cytological Conditions in the Pig, *Sus scrofa* 35
General Considerations 36

INTRODUCTION.

The chromosomes of the germ cells have received considerably more attention than have their descendants in the body and our knowledge of the behavior of these chromosomes is based on comparatively few studies of somatic cytology. During the process of karyokinesis in the body tissues the chromosomes may be studied, thus affording a basis for a comparison of their general characteristics and behavior with those of their ancestors or predecessors in the reproductive glands. Such investigations may eventually give an insight to some of the problems of development, including the function or activities of the chromosomes, and may also aid in strengthening the position taken in regard to these bodies by students of gametogensis.

Cytologists have demonstrated that in any given species the chromosome number is constant and that in the diploid complexes of the germ and body cells the chromosomes exist in pairs. For a given species the chromosomes are frequently readily recognizable by characteristic structure and behavior. This numerical and morphological constancy has led to the theory of the individuality of chromosomes and is now so well supported by many observations that it may be said to be a part of the creed of modern biology. Indeed our knowledge has reached the point where numerical variations are not considered as prima facia evidence against the theory, but as another manifestation of an imperfectly understood organization.

The following report deals with the chromosomes in the so-

matic cells of the evening primrose, *Œnothera scintillans*, de Vries, and of the pig, *Sus scrofa*, in which the diploid chromosome number of the soma was found to be variable. The bearing of these facts upon the individuality theory is indicated.

The Cytological Conditions in *Œnothera scintillans*.

The somatic, or diploid, number of chromosomes in *Œnothera scintillans* is fifteen. In material collected from three generations of these plants variations of this number have been found to range from fifteen to twenty-one. This diversity in number is not due to additions to the chromosome complex but to the fragmentation of certain individuals. From a preliminary study of the root tips of a fourth generation of *O. scintillans* I am inclined to believe that here the increase in chromosome number is not as great as just indicated. Breaks or clear areas occur in certain chromosomes which make what may be a single chromosome, appear as two. In my earlier material the possible relationship of chromosome portions on either side of the clear space was not so obvious as in the latest specimens. The last root tips collected present these portions in such perfect alignment as to suggest that they are a part of a common body. The clear areas are variable in extent and in certain cases one portion of the chromosome may be turned at right angles to the other. In a few instances the separation seems to be complete. The blank spaces in these "hyphenated" chromosomes may well be weak spots where under certain conditions breaks occur. As I have not yet determined the nature of these clear intervals in the chromosomes, for the sake of analysis, in this paper, all the separate chromatic bodies are considered as individual chromosomes.

Since the chromosomes are small and have no marked characteristics by which they may be recognized, measurements have to be resorted to. Some interesting results have been the outcome.

1. The total of the chromosome lengths of any complex whether it has the normal or higher number of chromosomes is the same. This indicated that no chromosomes have been added.

2. When the fifteen chromosomes of the normal complex are arranged according to decreasing length it is found that they

exist in a series of seven pairs plus one unpaired chromosome. Each pair differs from the pairs longer and the next shorter pair by almost precisely the same amount—nine per cent.

3. Since such exact relations exist between the normal pairs alteration in any of the chromosomes would destroy these conditions. This was found to be true in cells possessing more than fifteen chromosomes. Comparison with the standard lengths and relationships determined for the chromosomes of the normal complexes made it possible to reunite, with fair accuracy, the fragments to the chromosomes from which they had broken. This reunion has shown that it was chiefly the longer chromosomes that were concerned in fragmentation.

4. In the germ cells, the telophase chromosomes of the first maturation division are favorable for study. Two classes of cells are produced by this division, one of which has seven and the other eight chromosomes. This is due to the fact that the unpaired, and smallest, chromosome passes to one pole undivided. When the two complexes are measured it is found that total length of their chromosomes differ exactly by the length of the eighth chromosome, or the unpaired element. The same percentage of length difference exists between the individual gametic chromosomes as between the somatic pairs showing the two sets to be entirely comparable.

The Cytological Conditions in the Pig, *Sus scrofa*.

The work on the cytology of this mammal was made possible through an improvement in the preservation of the chromosomes which gave, I believe, the first true picture of the conditions in the cells of this group. Exception possibly should be made to the material used by Winiwarter in his studies, although it seems that the clear complexes were not as numerous as the other kind, whereas in the present specimens good mitotic figures were exceedingly abundant.

In the pig, I have found essentially the same cytological phenomena as in *Œnothera scintillans*. The spermatogonial chromosome number is forty and this appears to be constant. In the somatic cells the number ranges from forty to fifty-eight. As in the plant the extra number is caused by fragmentation and not

by additions, as the total length of the chromosomes of all complexes, regardless of the number of elements involved, falls within the total length limits established for the spermatogonial chromosomes. Furthermore, it appears to be the chromosomes of the long end of the series that are breaking up. In the normal complexes the chromosomes exist in pairs (not obvious until arranged according to length) and there is a relatively constant length difference between pairs. The normal complexes of the soma and of the spermatogonia agree in all particulars.

The fragments are not lost but continue as a part of the complex. They are never aligned with other chromosomes, as in *Œnothera scintillans*, in such a way as to suggest that they may be a part of those chromosomes separated from them by a clear space. The fact that in complexes made up of more than forty chromosomes fewer V-shaped elements are found led to a tentative suggestion in a recent paper (Hance, '17) that breaks occurred at the point of fibre attachment, *i. e.*, at the apex of the V. This gains some confirmation from the fact that the fragments (those chromosomes lying below chromosome 40 in the length series) are approximately the length of the short arm of the V.

General Considerations.

It is not possible, at present at least, to state the cause of fragmentation. Since the condition was found in a plant known to be unstable and in the pig which may very likely be genetically impure led to the suggestion (Hance, '17) that the phenomena might be in some way associated with hybridity. Such close breeding forms as the grasshoppers and the mosquitos are known to have a constant number of chromosomes in their body cells. There seems to be other evidence sufficiently strong and convincing on the other side to make the above idea a doubtful possibility rather than a reality. The chromosomes which break up may be multiples such as McClung and others have described but I think that there is considerable evidence against this. There is not enough known of somatic cytology, at present, to permit any sweeping generalizations.

Certain facts of chromosome behavior which are of general interest were disclosed through the employment of metrical

methods of analysis. The total of the chromosome lengths of any two complexes in any one form may be quite different and yet precisely the same relationship between pairs is maintained. If the total lengths of the chromosomes of certain cells differ, for example, by ten per cent. then the reduction of each chromosome of the "longer" cell by this amount will faithfully reproduce the length conditions in the shorter complex. In other words, anything influencing the length of the chromosomes in a cell will affect all proportionally. Consequently it can be readily understood that though the total lengths may vary considerably the interpair relationship remains constant. Data in support of these statements has been recently published (Hance,'17).

Since only the organization of certain chromosomes, and not the amount of chromatin, is disturbed by fragmentation it does not seem to me that the individuality is lost. It is true that certain chromosomes no longer exist as they were but since all the parts are retained intact in the complex the individuality hypothesis, as understood today, would seem sufficiently broad to admit these cases.

The discovery of the relationships of the chromosomes during the analysis of the unusual conditions just reported, is, I think, of considerably greater importance than the conditions themselves. Chromosome pairs exist in the soma and in the germ cells which are entirely comparable. When arranged according to length these pairs form a graded series. Between each pair there is a constant difference in length. These relationships exist in several other forms I have studied and appear to be fundamental. Besides presenting several new problems of chromosome behavior and organization, the constancy of these interpair relationships affords a check on the accuracy of cytological work and a basis for comparison with the work of others.

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Other papers of importance upon this subject are listed in the bibliographies of these two publications.

THE RELATION OF RHYTHMS AND ENDOMIXIS, THEIR PERIODICITY AND SYNCHRONISM IN PARAMECIUM AURELIA.

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Periodic nuclear reorganization or endomixis in *Paramecium*, noted by Hertwig ('88–'89) and since then by many other investigators, and described in detail by Woodruff and Erdmann ('14), is of great interest because of its bearing on questions of parthenogenesis, immortality and nuclear functions in Protozoa primarily, and secondarily in organisms in general. In a series of recent papers Woodruff and Erdmann ('14, '16) and Woodruff ('17, *a* and *b*) have advocated the view that it is a sine qua non in the life of *Paramecium*, that it is a rejuvenating process analogous to the similar process in conjugation, and that it usually occurs at the low point in the division rate between two "rhythms," the time interval of which is a fixed quantity inherent in the species and which cannot be modified by the external environment.

Kasanzeff ('01), Popoff ('09), Sun ('12) and myself (Young, '17) have shown that external agents may serve to induce endomixis, and my own experiments show that the frequency of its occurrence may be increased in this way. In my paper (*l. c.*, p. 52) I have stated that "It is more or less cyclical in character and probably has a rejuvenating function. It is not however a necessary accompaniment of temporary depressions and may not be followed by rejuvenation." I have pointed out several cases of the occurrence of low division points without endomixis in Woodruff's and Erdmann's experiments, as well as in my own. I have also indicated one case in their experiments and one in my own in which the process was associated with a rising, rather than a falling division rate; and have shown that in some instances endomixis is not followed by rejuvenation.

In the graphs of Woodruff's recent papers ('17, *a* and *b*) are shown several instances of low division points without endomixis, viz., in ('17*a*), Fig. 2, periods 1 and 12; Fig. 3, periods 1, and 5 and 6; Fig. 4, periods 2 and 11; Fig. 5, period 19; and in ('17*b*) Fig. 1, A, period 22; AE, periods 2 and 13; O, period 11; B, periods 22 and 23, and 27; Fig. 2, A_T, periods 3, 19 and 25; AE_T, periods 12, 17, 19, 25 and 27; O_T period 12 and B_T period 23, etc.

In Fig. 4 ('17*a*) are shown two low points without endomixis which appear to me essentially similar to those at which this process occurred (cf. per. 2 and 11). If the latter mark the division point between rhythms, why not the former? In this connection I wish to call attention to the author's words on p. 55: "During this time nine rhythms occurred at each of which endomixis was observed. *It is hoped that readers who may possess doubts concerning rhythms and endomixis will carefully consider this graph.*[1]

Regarding periods 19 and 25, A_T, Fig. 2 ('17*b*) and similar periods elsewhere Woodruff says (p. 446): "A second point of importance is that the 'rhythms' in the division rate are to a certain extent independent of endomixis—that is of the definite series of nuclear phenomena—because the rhythms persist for a while in the absence of the morphological changes. . . . So, it seems to be a more or less academic question whether rhythms and endomixis are independent. Certainly the rhythms occur for a while without endomixis in *sensu stricto*—but *in all such cases*[2] the culture has died before very long." While this statement applies to *some* of Woodruff's experiments it by no means applies to all. Many of the cases cited above, and many other cases shown in his graphs which I have not cited, besides several cases in my own experiments noted by me (*l. c.*, p. 47) disprove it. See for example his own statement (*l. c.*, p. 446) referring to subculture B_T, Fig. 2, "From the character of the curve it would be expected at period 23, but it was not observed."

This leads us to the question of the meaning of "rhythm" and of the influence of the graph in determining the same. Accepting Woodruff's definition of the former as " a minor periodic rise and

[1] Italics mine.

[2] Italics mine.

fall of the fission rate, due to some unknown factor in cell metabbolism, from which recovery is autonomous" ('05, p. 604), we must consider the then "unknown factor," which virtually makes "rhythm" synonymous with inter-endomictic period, a sense in which the term "endomixis was *not* used . . . when first employed by Woodruff and Erdmann" (Woodruff, '17*b*, p. 447). But if we do not consider this factor in defining "rhythm," its definition becomes a wholly arbitrary matter dependent upon the character of the graph employed, which as Woodruff and Erdmann ('14, p. 447) have shown is at best an artificial means of representing the history of the race in question, and which tends to emphasize or obscure the rhythms as the average period is shortened or lengthened.

While cessation of endomixis was followed by death in two of Woodruff's experiments (A_T and AE_T, '17*b*), both my own and Woodruff's experiments show many cases in which the process occurred shortly before the death of the race in question—see my Figs. II, VIII, VIII*a*, X*a*, etc., and Woodruff's ('17*b*) Figs. 1, O, and 8, B_s and M_s. It is of course entirely possible that death in the former cases resulted from cessation of endomixis, and in the latter from some other cause, but on this question the experiments throw no certain light.

Woodruff's recent work has further disclosed several additional cases of the exceptional occurrence of endomixis at a high, or rising, rather than a low, or falling point in the division rate. I have previously noted two of these exceptions (*l. c.*, p. 47) one in my own experiments and one in those of Woodruff and Erdmann (1914), to which may be added here the occurrence of the process at periods 68 O, 81 B, Fig. 7; 61 A_s, 82 and 86 O_s, 70 B_s, 70 and 82 M_s, Fig. 8 and 76 M, Fig. 12.

In most of Woodruff's graphs the variations are too irregular or the period too short to show any regular rise and fall in the division rate over considerable periods, but in one case (*A*, Fig. 9, p. 455, 17*b*) such alternate periods are clearly shown, without however any evident correspondence between endomixis and the periods of low and high division rate. An endomixis is as liable to occur at a high point as at a low point on the curve, neglecting the minor fluctuations of a few tenths of a division which mark

the intermediate points of the "rhythms" in this curve. The same is true of other curves in which an alternate rise and fall in the division rate over long periods is not evident. Take for example the graph of line *M* in Fig. 12 (*l. c.*, p. 458). Here there is no relation whatever between absolute division rate and endomixis, the process occurring four times during division periods of 2 + per day and five times when the division rate was 2 − per day, the only relation between endomixis and division rates being evident in the minor fluctuations or "rhythms."

In view of these results, I cannot find any constant correlation between endomixis and division rate in *Paramecium*.

Why then is it that endomixis is *usually*, though not always, accompanied by a temporary reduction in the division rate? I believe the latter to be consequent to the former. During endomixis profound chemical and physical changes are taking place in the cell, of whose nature we are at present ignorant, but which must necessarily exercise a great influence on metabolism and growth, and would naturally tend to temporarily diminish the division rate. Those exceptional cases in which endomixis occurs at a high, or rising point in the division rate may perhaps be attributed to some extrinsic stimulus in the culture medium, tending to reverse the usual sequence of events.

In his various papers on the subject of "rhythms" in Protozoa, Woodruff maintains a more or less regular periodicity in their occurrence (25–30 days or 40–50 generations in *Paramecium aurelia* and 50–60 days or 80–100 generations in *P. caudatum*). In his most recent paper ('17*b*, p. 461) he states that "General changes in the environment of the animals . . . do not permanently modify the length of the rhythm or the time between successive endomicitic periods which is characteristic of the species." But (p. 462) "it has been found that the generation periodicity . . . may be modified to a considerable degree by the culture conditions which lower the division rate. In other words, the rhythm appears to be more susceptible of modification in regard to generations than time. As has been previously noted, this is a surprising result, since a profound reorganization process such as endomixis must be closely related to the general metabolism of the cell and this is expressed to a large extent in growth and reproduction."

With the second of these conclusions I am wholly in accord,[1] agreeing as it does with the results of my own work (*l. c.*). The first is not however in accord with my experiments and cannot I believe be verified by a critical reading of Woodruff's own results. That the periodicity of the rhythms is subject to greater variation than he admits may be deduced from his own data. In order to show this I have compiled a table based upon the graphs and tables of several of his own and Miss Erdmann's recent papers, the exact source of the data being indicated in every case.

In this table are shown the number of five-day periods, generations and variation percentages of the rhythms in various lines of *Paramecium aurelia*, based on data the source of which is indi-

1.	2.	3.	4.	5.	6.	7.	8.	9.
5	4.7	+ 6	64	47	43.5	+ 8	27	W & E ('14) line VI, Table 1 and text fig. 16.
3		−36		36		−17		
5		+ 6		43		− 1		
6		+28		48		+10		
				79	59.5	+33	66	*idem*, line VI*h*, Table 1.
				40		−33		
8[4]	6.5	+23	76	75[2]	62.5	+20	48	*idem*, line III, Table 2 and text fig. 17.
4[4]		−38		49[2]		−22		
5		−23		48		−23		
9		+38		78		+25		
4	4.7	−15	85	53	55.9	− 5	104	W ('17*a*), fig. 4.
6		+28		80		+43		
5		+ 6		56		00		
6		+28		60		+ 7		
6		+28		70		+25		
2		−57		22		−61		
4		−15		47		−16		
5		+ 6		59		+ 6		
2	5	−60	100	27	47.8	−44	124	*idem*, fig. 5.
7		+40		86		+80		
4[3]		−20		30[2]		−37		
7[3]		+40		53[2]		+11		
5		00		43		−10		

[1] Using "rhythm" as synonymous with inter-endomictic period.

[2] According to Woodruff's data there is a little uncertainty regarding the occurrence of endomixis at generation 4140 in line III. I have based my data on the assumption of its occurrence at that time which he states was "almost positive" (*l. c.*, p. 470).

[3] Uncertainty exists in Woodruff's data regarding the occurrence of endomixis at period 14. My table is based on the assumption of its occurrence there, which he considers very probable judging by "the character of the curve" (*l. c.*, p. 56).

1.	2.	3.	4.	5.	6.	7.	8.	9.
5	5.5	− 9	72	34	42.4	−20	102	W ('17*b*) figs. 1 and 7, line A.
6		+ 9		54		+27		
6		+ 9		60		+41		
7		+27		69		+63		
4		−27		45		+ 6		
5		− 9		43		+ 1		
7		+27		31		−27		
5		− 9		33		−22		
8		+45		45		+ 6		
4		−27		26		−39		
4		−27		26		−39		
5	5.2	− 4	38	53	54.5	− 3	53	*idem*, line O.
6		+15		71		+30		
5		− 4		60		+11		
4[1]		−23		50[6]		− 8		
6[1]		+15		47[6]		−14		
5		− 4		42		−23		
4	5	−20	80	78	55	+42	97	*idem*, fig. 7, line B.
7		+40		59		+ 7		
6		+20		58		+ 5		
3		−40		25		−55		
2	4	−50	75	10	12	−17	50	*idem*, fig. 8, line A_8.
4		00		15		+25		
5		+25		14		+17		
5		+25		9		−25		
5	4.7	+ 6	21	36	30	+20	33	*idem*, line O_8.
5		+ 6		28		− 7		
4		−15		26		−13		
3	4	−25	100	29	31.6	− 8	82	*idem*, line M_8.
2		−50		17		−46		
6		+50		37		+17		
5		+25		43		+36		
4		00		32		+ 1		

[1] I have considered the two endomixes shown on the graph at periods 61 and 63 as a single event, which I infer to be the case from the table on page 452.

cated as follows: W = Woodruff, W & E = Woodruff and Erdmann, with the date of publication following the author's initials in each case.

Columns 1 and 5 show the number of five-day periods and generations respectively, columns 2 and 6 the average of each, columns 3 and 7 the variation percentages based on the average, columns 4 and 8 maximum differences of the latter, and column 9 the source of the data. In every case "rhythm" is the same as inter-endomictic period.

The above data are sufficient I think to refute Woodruff's

claim as to rhythmical constancy in *Paramecium*. Certainly variations of from fifteen to twenty-five days in periods never exceeding forty-five days are scarcely in accord with his statement " . . . that once established the rhythmic period characteristic of the species is maintained within rather narrow limits " ('17*b*, p. 452).

In both his joint paper with Erdmann ('14) and his most recent one ('17*b*) Woodruff cites Tables 1, 2 and 3 ('14, pp. 462–463) as evidence of synchronism of endomixis. Again a careful reading of these tables fails to support his contention. Of twenty-one endomixes shown in Table 1 for example, fifteen show close or fairly close synchronism, but of these four occurred in lines which had but recently been branched off from the parent lines, and hence had not yet had opportunity to vary to any considerable degree from the condition of the latter. I refer to the endomixis at generation 4101 in line VIC and to those at about generation 4180 in VId, e, and g. If we reject these as proving nothing one way or the other, we find that out of seventeen endomixes eleven support Woodruff's claim, while six fail to do so. The exceptions in other words are too numerous to "prove the rule." Of especial interest are those lines which, while only recently started, show a marked divergence from the parent stock, showing that the supposed synchronism may be disturbed within a very few generations (VIb–4092, VIf–4189). This fact has been noted by Woodruff and Erdmann ('14, p. 461), but passed by with the remark that it " . . . has no significance from the standpoint of the synchronal appearance of the process in sister *lines* . . ." Further evidences of lack of synchronism are afforded by line IIIa, generation 4271, Table 2 and line V, generation 4115, Table 3.

Synchronism is better shown in *P. caudatum* (cf. Table 1, p. 69, Woodruff and Erdmann, '16) but even here there are a few exceptions (cf. lines I and III, generations 1–9, column 1). Other exceptions are shown in line Ica, generations 180–270, column 3, and in line Ie generations 270 to 450, columns 4 and 5, in Table 3 (*l. c.*, p. 71).

Regarding the synchronism of the same races of *Paramecium* when bred in different environments, Woodruff's table ('17*b*,

p. 455) shows that of seventeen endomixes in the four pairs of cultures to be compared,[1] the synchronism was exact in only six cases, approximately exact (*i. e.*, within one five-day period) in six and inexact (differing by at least two five-day periods, which in a total interval of never more than seven and seldom more than five such periods is considerable) in five. These facts, taken from Woodruff's own data, hardly seem to prove his claim regarding the maintenance of rhythmical constancy and synchronism in *Paramecium* in either the same or different environments.

Were this negative evidence the only refutation of his contention however I should hesitate to urge it too strongly. But I have positive evidence from my own experiments leading to directly opposite conclusions.

The graphs in my previous paper (*l. c.*) show numerous cases of irregularity in the time period of endomixis. In experiment 1 (Figs. 1 and 1*a*) for example line I shows a fairly regular endomictic periodicity with an average interval of eighteen days,[2] while line Ia, branched off from I on 5/8 was carried for a period of forty-two days without any endomixis whatever. Here the disturbance of the period is obviously due to changed environment; I being carried for the most part in stale, and Ia in fresh culture media. Experiment 2 shows that, whereas the first three endomicitic periods in line II, branched from I on 2/8, agree fairly well with the corresponding periods in I, following the endomixis on 3/5 no others occurred until 5/13, a period of sixty-nine days. This change in period is probably traceable in part to changed environment, as suggested in my previous paper (*l. c.*, p. 39). It is probable however that some factor inherent in the cell itself is also in part responsible since the line did not undergo the *urea* treatment until 4/21, forty-seven days after the last previous endomixis occurred. Whatever the cause however the experiment shows that marked variations in endomictic periods do occur.

Both lines IIa and III which are derivatives of line II, while showing essentially the same periodicity with respect to each

[1] Not counting those at period 59 which may have been induced by the sudden change to the stale culture medium.

[2] Omitting the doubtful record on 3/16 cf. footnote 5, p. 39 (*l. c.*).

other, show a periodicity differing from both the earlier and later period in the parent line II; while lines IV and VI, derived from II on 4/13 and 4/15 respectively, show periodicities differing from each other and from those of all three preceding lines (II, IIa and III). In line XII marked variations in periodicity are shown.

Since these experiments were not designed to test the periodicity of endomixis it is difficult in most of the above cases to determine the cause of the variations noted, whether extrinsic (environmental) or intrinsic. In the following experiments however we have very clear evidence of the influence of environment, in this case temperature, in altering the endomictic period. Comparing the length of period in lines XIIa and b with that in the parent line XII for example it is seen that increasing the temperature reduced not only the generation period, but also the time period, from an average of twenty-five days in XII to one of seven in XIIa and of twelve in XIIb. Even more striking evidence of the same character is furnished by experiments 14, 15 and 16.

Notwithstanding Woodruff's emphasis upon the constancy of the time period of endomixis, in contrast to the generation period, which latter was markedly affected by changed environment; it is reasonable to assume that the latter is the more important, indicating as it does the amount of growth and hence the metabolic activity of any period. He himself says (*l. c.*, p. 458), "This is a most surprising result, because such profound reorganization phenomena as are involved in endomixis must have a more or less definite relation to the physiological activity of the protoplasm, the best criterion of which is generally considered to be growth and reproduction as indicated by the division rate. More experiments obviously are needed to resolve this 'time factor' into its significant elements." In the absence of further experiments and in the light of the contradictory results of my own experiments and of many of those of Woodruff himself as analyzed above, I would suggest that the time synchronism, when present, is a result of generation synchronism; and that in those cases in which the latter, but not the former was modified by environment, the constancy of the former in those cases in which it was constant or approximately so (twelve out of seven-

teen) was due to chance, and not to any inherent constancy in the periodicity itself.

Undoubtedly there is more or less regularity in endomixis, just as in any other physiological process (cell division, reproduction, respiration, excretion, etc.); but to prove that this has a fixed time periodicity, which, unlike that of other physiological processes, cannot be modified by environment, will require much stronger evidence than Woodruff has as yet presented. His own data are contradictory and my results point very strongly to an opposite conclusion.

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[1] Not seen by me.

SELF FERTILIZATION IN THE AIR-BREATHING POND SNAILS.

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The author has already shown that under certain conditions self fertilization is possible in the common pond snail *Lymnæa columella Say*.[1] In this paper he wishes to point out that it is common in species representing most of the important genera of the order Basommatophora, the order which includes the air-breathing pond snails.

The criteria on which self fertilization in *Lymnæa columella* has been determined is as follows: (1) The snails are hermaphroditic and eggs and sperm are ripe in the ovotestis at the same time, (2) self fertilization is mechanically possible and self copulation has been observed, (3) two polar bodies are found on the eggs produced by snails isolated from their fellows from the time of hatching, (4) normal parthenogenesis is unknown in the Mollusca. The chances therefore that the phenomena observed is parthenogenesis and not self fertilization is very nearly nil.

In every case the following method was used. A snail was isolated and placed in a jar holding from 300 to 500 c.c. of filtered pond water. A pinch of dry garden soil was added[2] together with a dry leaf or two from a Carolina poplar tree for food. When the snail laid eggs and these hatched, each young one, when a day or two old, was isolated and placed in a jar under the conditions stated above. If these young snails grew to adult size and laid eggs which in their turn developed, then self fertilization was assumed.

The material used was *Lymnæa columella* from around Philadelphia, *Lymnæa columella* from Chicago, *Lymnæa humilis modicella* Say, from Philadelphia ponds, *Lymnæa catascopium* Say, from the Delaware River near Torresdale, Pa., *Lymnæa*

[1] Colton, Proc. Acad. Nat. Sci. Phila., May, 1902.

[2] Colton, Proc. Acad. Nat. Sci. Phila., July, 1908.

reflexa Say, from Chicago, *Lymnæa auricularia* Linne, from a pond near Oak Lane, Philadelphia, *Physa heterostropha* Say, from Philadelphia, *Planorbis exacutis* Say, from Philadelphia, *Planorbis parvus*, Say, from Philadelphia, and *Ancylus fluviatilis* from the same place. In every case isolated snails laid fertile eggs.[1]

That self fertilization is not normal when a number of snails are together in an aquarium may be inferred from the fact that one so often observes two snails in copulation. The author has even observed different species in copulation, *Lymnæa columella* and *Lymnæa humilis modicella*. In the case observed the mass of sperm that had been injected into the vagina of the snail acting as the female was soon expelled into the water where it fell to the bottom as a mass of whitish mucus. When this was examined under the microscope it was seen to contain spermatozoa. All attempts at crossing species proved failures.

The significance of the fact that self fertilization is possible in the air-breathing pond snails is that pure lines can be established in this group of animals in the same sense as in plants. That continued imbreeding of this closest sort does not cause the strain to become weak and finally extinct is proved by the fact that the author has one line of *Lymnæa columella* which has passed through thirty one self-fertilized generations.

[1] The writer wishes to thank Dr. H. A. Pilsbry for his kindness in identifying the snails mentioned in this paper, and Mr. E. H. Hand for supplying material from Chicago.

THE EFFECT OF RADIUM RADIATIONS ON THE DEVELOPMENT OF CHÆTOPTERUS.

CHARLES PACKARD.

Introduction.

The experiments described in this paper show that the egg of *Chætopterus*, if radiated for an appropriate length of time before insemination will be injured to such an extent that its nucleus plays no part in development. Nevertheless the sperm nucleus divides normally; development is therefore androgenetic. The history of the egg nucleus from the time of insemination until it is absorbed into the protoplasm can be followed in detail.

Similar experiments have been performed by Gunther Hertwig ('11) on the frog's egg. He observed that if the eggs, after a brief radiation, were inseminated with fresh frog sperm, the amount of injury appearing in the embryos is proportional to the length of exposure. But if the eggs are radiated for more prolonged periods, cleavage becomes fairly normal, and the embryos, somewhat retarded in their rate of development, grow into swimming larvæ. These larvæ are normal in shape but are smaller than the controls and not as active, in these respects resembling parthenogenetic larvæ. He also observed that the nuclei of the cells in the embryos developing from eggs radiated for considerable periods are about one half of the size of the nuclei found in the controls. Since the size of the nucleus, according to Boveri, varies with the number of chromosomes contained in it, the inference is that these embryos have developed under the influence of the sperm nucleus and are therefore androgenetic. The egg nucleus has been entirely prevented from taking part in development. Cytological proof of this critical point was lacking.

The experiments of Paula Hertwig ('16) on the eggs of *Triton* do not wholly confirm the results just described. Exposures of the eggs for 5 to 30 minutes to a preparation of 51 mg. of radium

bromide, previous to insemination, resulted in embryos which were both normal and abnormal. In general there was little difference between the results obtained from the short and from the long exposures. Measurements of the nuclei in the radiated embryos showed that they were approximately one half the normal size and the development was presumably androgenetic. The actual number of chromosomes could not be counted with certainty. Hertwig states that "a certain answer to this question (of the exact number of chromosomes) could be furnished only by a study of the eggs during their early development. Since this is impossible, owing to practical difficulties which the material presents, the question must remain unsolved."

In the reverse experiment, in which frog sperm was radiated, it was found by Gunther Hertwig ('11) that the same developmental conditions obtained which he found when the egg was radiated. Normal eggs fertilized by sperm which had been radiated for short periods gave rise to abnormal embryos. But if the sperm is radiated for a long period the embryos developed normally. Later he repeated this experiment on the sea urchin and found that although the intensely radiated spermatozoon can penetrate the normal egg it fails to develop within the egg, and brings about an abnormal type of cleavage. The egg nucleus may divide by itself, sometimes normally, but more often irregularly, as though the sperm had acted as a mechanical hindrance to orderly cleavage. When the sperm had not thus interfered in the first mitosis it usually fused with the nucleus of one of the blastomeres, thus causing abnormalities in the descendants of that cell. In all of these cases the sperm aster developed normally, the centrosome divided, and the daughter centrosomes became the centers of the first cleavage figure. This fact lead Hertwig to doubt Boveri's conception of the centrosome as a permanent cell organ, and to incline to Lillie's view that it is a result of the interaction of the sperm and the egg protoplasm.

Material and Methods.

The eggs of the tubicolous annelid, *Chætopterus pergamentaceus*, are ideal for the type of experiments described in this paper. They can be obtained fresh and in sufficient quantities,

polyspermy is rare, and the control eggs develop with perfect regularity if kept under appropriate conditions. The number of chromosomes is small, the haploid number being nine, and the achromatic structures are unusually large. The most valuable feature is the fact that the unfertilized eggs, when placed in sea water, proceed in their development as far as the metaphase of the first maturation division, at which time further development ceases. Now it has been shown (Packard, '16) that chromatin, when in its most condensed stage at the time of the metaphase, is particularly susceptible to radium radiations. A short exposure therefore is sufficient to injure the chromatin without harming the protoplasm. This is an important point, for I have shown (Packard, '15) that prolonged radiations injure the protoplasm and bring about a very abnormal type of development quite different from that which follows the injury of the chromatin alone. A further advantage possessed by these eggs is their extremely labile condition. As Miss Allyn ('12) showed, they can be stimulated to develop parthenogenetically by a great number of agents. I have found that prolonged exposures will not prevent it from developing when fertilized by a normal sperm.

The metaphase of the first maturation division in the egg of *Chætopterus* is reached about twenty minutes after the unfertilized eggs are put in sea water. In carrying out an experiment I allowed the eggs to remain for this period in sea water and then exposed them to the radium. A quantity of eggs is gathered into a compact mass in the center of a watch glass containing a measured quantity of water. The tube containing the radium bromide, having the strength of 50 mg. of pure bromide, is now held over the center of the mass of eggs at a distance of $\frac{5}{16}$ inch above it. It is obvious that the eggs did not all receive exactly equal amounts of radiation, but the difference in amount which each received is very slight.

The alpha rays are not able to penetrate the thin walls of the glass tube in which the radium salt is held and are therefore not responsible for any of the effects obtained. Probably some of the slower beta rays are also cut off by the glass or by the sea water over the eggs; but the great majority of beta rays and all of the gamma rays are able to reach the eggs.

The unfertilized eggs were exposed for periods varying from 5 to 135 minutes, after which they were inseminated with *Chætopterus* sperm, and their behavior in the formation of polar bodies and in cleavage recorded. In all of these experiments controls were kept, made up from the same lot of eggs and subject to the same temperature conditions.

For cytological study the radiated eggs were killed, at various times after insemination, in Meves' fluid, Boveri's picro-acetic, and in Bouin's fluid. The last named reagent proved to be the best for most purposes. It acts rather destructively on the centrospheres, but is otherwise very good.

The Normal Development of *Chætopterus*.

The process of maturation and fertilization in *Chætopterus* has been described by Mead ('97). When the eggs are taken from the genital segments of the female and put into sea water the wall of the germinal vesicle breaks down. Fibers from two well-developed egg asters grow in toward the chromosomes which gradually assume an equatorial position. The spindle thus established moves toward the animal pole, rotating as it goes, until it comes to rest at right angles to the periphery of the egg. The chromosomes are already split, being tetrads at this time, but no actual separation occurs until after the sperm has penetrated the egg. In this condition the egg remains until fertilized.

As soon as the sperm has penetrated the egg the first polar body is quickly extruded, about 15 minutes after insemination. The second polar body is given off about 10 minutes later. Each chromosome remaining within the egg bends into a V shape and the entire group of nine gathers about the remaining egg aster. Before the group begins to move inward toward the center of the egg each chromosome becomes vesicular. During the inward journey the separate vesicles fuse to form a single large egg nucleus. The egg aster disappears when the fusion of the chromosomal vesicles takes place.

The sperm head does not begin to grow until it has penetrated the egg for some distance. But before its growth commences a small sperm aster with a centrosome appears in front of it. As the sperm moves inward the centrosome divides, each daughter

centrosome being surrounded by a well-marked aster. Finally the sperm grows into a vesicular form resembling the egg vesicle with which it soon comes in contact. Actual mingling of the chromatin elements does not occur until the metaphase of the first cleavage. Up till that time the chromosomes from each nucleus can easily be distinguished. Before the chromosomes have condensed into their characteristic shape there can be seen in the cleavage nucleus a number of chromatin nucleoli, probably derived from the chromosomes. During cleavage these lag behind, staying in the cleavage plane. The achromatic stuctures are very prominent, the asters remaining even after the completion of cleavage.

Observations on the Living Eggs.

To test the effect which gradually increasing amounts of radiation will produce in the rate of cleavage and in the production of abnormal cleavages it is necessary to employ throughout any one experiment eggs taken from a single female, for it was found that eggs from different specimens varied in the rate of cleavage and in other ways. In each experiment a sufficient quantity of eggs was exposed, and from this number a few were taken off at 10-minute intervals and fertilized in finger bowls. The proportion of cleaving eggs, compared with the controls, and the number of abnormal cleavages were recorded. This experiment was repeated on different days, and with eggs derived from different females.

It was found that brief radiations do not affect the rate at which the polar bodies are extruded. Indeed, prolonged radiations have little effect, for only in a few instances could any unusual condition be found. A cytological study showed that as long a treatment as 80 minutes causes very few abnormal polar divisions. Cleavage is much retarded, the most pronounced retard being seen in eggs radiated for 30 to 40 minutes previous to insemination. Longer exposures result in a more normal rate. This unexpected result is due to the fact that eggs which have been radiated for a long time are almost all polyspermic and such eggs always divide rapidly, sometimes ahead of the controls, and always into three or more parts.

The significant feature in these experiments is the sudden drop in the rate of cleavage seen in eggs radiated for periods up to 40 minutes. The explanation for this phenomenon is to be found in the behavior of the egg nucleus during maturation and subsequent growth, and in the behavior of the sperm nucleus during cleavage.

Cytological Observations.

A cytological examination of eggs exposed for periods of 15 to 35 minutes previous to insemination shows that the first effect of the radium radiations is on the chromatin; the protoplasm is not visibly affected. The abnormal behavior of the egg nucleus is perfectly apparent, but even prolonged radiation produces no visible change in the protoplasm. Without doubt the latter is affected, for polyspermy becomes more and more common as the duration of the exposure is lengthened. In *Nereis*, polyspermy under these conditions is due to a weakening of the egg membrane and perhaps to a partial liquefaction of the protoplasm. The same explanation may hold true for *Chætopterus* also.

All the eggs exposed for periods up to 35 minutes show the same types of abnormalities but in varying degrees, the more pronounced being found naturally in eggs exposed for the longer periods. The outstanding feature in the development of these eggs is the ever increasing tendency of the germ nuclei to remain apart. This is not due to any unusual development of the sperm; its centrosome divides normally, the asters appear, and the whole apparatus moves inward at the usual rate (Fig. 1). But the egg nucleus often remains close under the region of the polar bodies and may even be connected with them by a protoplasmic bridge in which chromatin threads can be distinguished. This phenomenon grows more common as the duration of the exposure is increased to 35 minutes. In this position it develops more or less normally, depending on the length of exposure. During cleavage the egg chromatin always remains separate from the dividing sperm chromosomes and can easily be distinguished from them because of its position and appearance. With this brief statement of the general course of development in the radiated eggs we may now examine in detail some of the conditions found during the various stages of maturation and cleavage.

After the sperm enters, the first maturation is completed normally and the chromosomes become arranged on the second polar spindle. There are very few exceptions to this, even in eggs exposed for a long time. Fig. 2 shows a condition which may be found occasionally in eggs treated for 80 minutes or more. It is evident that the whole egg, both chromatin and protoplasm, has been greatly disturbed, for the sperm after entrance has failed to develop normally. In the course of normal development it grows into a large vesicle by the time it has reached the vicinity of the egg nucleus, but in this case there is no evidence of any such growth. The tripolar spindle of the first maturation division indicates again how extensive is the injury to the egg. The extra aster may be derived from the sperm aster, or possibly both sperm asters are involved, one having fused with the egg aster; or the latter may have divided, while the sperm aster has not developed at all. The latter view is very likely correct for in other eggs similarly treated the sperm may be seen lying in the center of the egg without any trace of centrosome or asters. Whatever may be the right interpretation it is evident that the egg protoplasm has been injured to such an extent that the sperm cannot develop normally. The same phenomenon can be seen in the eggs of *Nereis* similarly treated. The chromosomes on the tripolar spindle show no signs of fragmentation but they have not divided, and some of them are irregular in shape. But in eggs treated up to 45 minutes the formation of the polar bodies and the development of the sperm is practically normal. The egg nucleus in a fairly large proportion of cases develops normally after the extrusion of the polar bodies and fuses with the sperm nucleus. The subsequent development of such eggs will be described later.

In a considerable number of eggs exposed for periods up to 45 minutes the egg nucleus does not behave normally. Instead of moving inward to meet the advancing sperm, it tends to remain in its original position under the polar bodies, there developing into the vesicular stage. Fig. 3 illustrates this condition. Here, although a part of the nucleus has moved inward, there still remains a part of it directly under the polar bodies, connected with the rest by a protoplasmic bridge. The distribution of the chro-

matin follows the shape of the nuclear vesicle; a little is still in the polar position. This condition prevents the egg nucleus from flattening out against the sperm nucleus. The latter is seen normally developed with its astral rays extending far out into the protoplasm.

A more pronounced case of the same sort is seen in Fig. 4. Here the egg nucleus has hardly moved at all but has developed in approximately its original position. Its inner end has come under the influence of a sperm aster, and some of its chromatin is evidently polarized. Its outer end is attached to the second polar body by chromatin threads and the whole nucleus has the appearance of being anchored to it.

Modifications of this condition are seen in the polyspermic egg shown in Fig. 5. Most of the egg nucleus has migrated away from the pole but a small piece about the size of a single chromosomal vesicle is still attached to the second polar body by delicate strands. Two sperms have entered; one of them has entirely fused with the egg nucleus, and the other is about to fuse. Only three asters can be found in this case. In Fig. 6 two sperms have fused with the egg nucleus which, as in the preceding case, is still attached to the polar body. Five asters can be found in connection with this cleavage nucleus. The number of instances in which three or five asters appear make it evident that the original egg aster may persist.

A final stage showing how the egg nucleus in the vesicular condition may fail to fuse normally with the sperm nucleus appears in Fig. 7. The latter nucleus is about fully grown and its asters are well developed. The egg nucleus is developed only in part. The two small vesicles contain numerous chromatin granules. Between them the remainder of the egg chromatin lies free in the protoplasm in the form of rods and spheres. These masses are not chromosomes for they have not the typical size or appearance. The curious positions which they have assumed indicate that the force drawing them inward is not normal.

Those eggs which were radiated for periods up to 25 minutes do not show many of the abnormalities just described. In such eggs the pronuclei develop normally to all appearance and fuse with each other. The first indication that any injury has been

done to the egg chromatin appears at the anaphase of the first cleavage. Fig. 8 shows an egg in which this is the case. There has not been a complete mingling of the two nuclei for the egg chromosomes are left behind in the future cleavage plane. They are for the most part normal in appearance. One is stretched out to an unusual length as though pulled at each end by a spindle fiber. If this is the case, the spindle-fiber attachment is unusual for here it is evidently terminal while in the normal chromosomes it is median. The nine sperm chromosomes in each group show the characteristic V-shape. Division is thus regular and haploid, and the injured egg chromosomes have not interfered with the orderly division of the egg.

A more noticeable injury to the egg chromatin is seen in Fig. 9. Here again the nuclei probably fused normally to all appearances. But at the anaphase, when the sperm chromosomes are moving towards the poles, the egg chromatin is left behind in a very confused condition. A few chromosomes have formed but they are noticeably beaded as though on the point of breaking up into fine granules. The remainder of the chromatin is in irregular masses. Possibly its position outside of the spindle accounts for the fact that the chromosomes show little sign of being drawn towards the poles. No spindle fibers can be found attached to it.

When the nuclei have failed to fuse, as shown in Figs. 3 and 4, the further growth of the egg nucleus is greatly modified. In Fig. 10 the chromatin is seen to be condensed into rods and irregular masses which lie wholly outside of the mitotic spindle. The rod-shaped masses are beaded. Only a few of the chromosomal vesicles could have migrated inward.

A polyspermic egg in which the same type of abnormality is found is shown in Fig. 11. From the distribution of the chromatin it may be inferred that this condition is a further development of such an egg as is shown in Figs. 5 and 6. It is interesting to note that the egg chromatin which shows the greatest sign of injury remains nearest to the pole. The more normal chromatin has migrated inwards. The sperm chromosomes which are distributed very irregularly are of normal shape and size.

These observations show that when the unfertilized eggs are radiated for periods up to 30 minutes before insemination, the

nucleus develops normally and fuses with the sperm nucleus. But during cleavage the egg chromosomes do not move together with the sperm chromosomes but lag behind in the future cleavage plane. Under the influence of longer radiations the egg is so affected that its nucleus is unable to move from its position under the polar bodies, but develops there more or less abnormally, and plays no part in the cleavage of the egg. In both cases the cleavage is regular and haploid. A prolonged radiation so affects the egg that the polar bodies may be formed abnormally or not at all; in such instances the sperm also fails to develop, though it can move up to the position of the polar spindle. Polyspermy becomes more and more frequent as the duration of exposure increases. This leads to abnormal cleavage into three or more cells.

Discussion.

The facts presented in the foregoing pages show that with increasing periods of exposure the number of diploid cleavages is lessened and the number of haploid cleavages, in which only the sperm nucleus takes part, is greatly augmented. Actual counts of sections of dividing eggs give a clear picture of the proportion which obtains between these two types. These counts are represented graphically in the accompanying text figure. The ordinates represent the percentage of eggs which cleave, and the abscissæ, the duration of the exposure in minutes. Polyspermic eggs were disregarded in making these counts because they belong to a different category of phenomena. The dotted line represents the percentage of eggs which divided regularly.

It is seen that brief exposures do not prevent eggs from dividing, but that exposures of 30 to 35 minutes bring about a marked decrease in the numbers which cleave. This relation was noticed in the living eggs, and the curve plotted from the latter data corresponds closely to that derived from a study of the sections. After this critical period the number of eggs which cleave regularly increases to about 70 per cent. and then, with prolonged exposures, gradually falls off until, after an exposure of 130 minutes, practically no eggs divide.

The other curves show why this sudden drop at the critical

period occurs. The number of eggs dividing with the full complement of chromosomes falls gradually up to the 30-minute period. Then it drops very suddenly so that after a treatment of 35 to 40 minutes very few eggs divide with the diploid number.

The proportion of eggs dividing wholly under the influence of the sperm rises rapidly at the critical period and remains high until prolonged radiation prevents cleavage altogether. Of the eggs which remain uncleft after the others have divided with either the diploid or the haploid number of chromosomes, most are in the condition shown in Fig. 9. These may divide at a much later time but I have no data on that point. It is evident, therefore, that during the critical period the egg nucleus has been injured to such an extent that it can take no part in cleavage, and in some way prevents or greatly retards the cleavage of the sperm nucleus. After that time it acts like a foreign body, exerting very little influence, if any at all.

The form of the curve shown in the dotted line of the text figure bears a striking resemblance to the curves figured by Hertwig ('11) in his article on the effect of radiations upon the unfertilized frog egg. In his experiments Hertwig exposed different lots of eggs to preparations of radium of different strengths, and then fertilized them with normal sperm. The criterion in estimating the effect of the treatment was the length of life of the embryos. In the three sets of experiments it appeared that with brief exposures the length of embryonic life varies inversely with the duration of the exposure. Thus, the eggs treated for 5 minutes before insemination with a preparation having the strength of 7.4 mg. of $RaBr_2$ lived for 5 days, at which age they were abnormal in many respects. An exposure of 15 minutes resulted in much greater injuries, and the embryos lived but 3 days. But with longer radiation, development was normal through the gastrula stage and the embryos lived 8 or 9 days. They were weak and small but fairly well formed. With weaker preparations of radium the results were less pronounced but of the same order.

Hertwig's explanation is that brief radiations injure the egg nucleus but do not prevent it from fusing with the sperm nucleus. However, the injury is sufficient to bring about the production of

abnormal nuclei in the later embryos, as he demonstrated cytologically. The longer exposures so injured the egg nucleus that it failed altogether to fuse with the sperm nucleus, and therefore played no part in later development. The embryos were therefore androgenetic.

He has brought forward no actual proof that this is the case in the frog egg, except the evidence furnished by measurements of the nuclei. Paula Hertwig has shown that when *Triton* eggs are similarly treated the number of chromosomes is less than the diploid number and probably the haploid number but of this she is not certain. But Hertwig's explanation is in part supported by the facts brought forward in this paper. A strict comparison cannot be made since his conclusions were based on the length of life of the embryos while mine have to do with the behavior of the germ nuclei up to the time of the first cleavage. But in all probability the same phenomena occur in both cases.

The results obtained in the reverse experiment, in which the frog sperm is first radiated and then added to fresh eggs, are of the same order as the foregoing. Up to a critical time the radiated sperm produces greater and greater abnormalities in the embryo; after that period, more intensely radiated sperm produces more normal embryos. The original experiment of O. Hertwig ('11) has been repeated by G. Hertwig ('12), Opperman ('13) and others with the same results. A cytological study of the eggs of the sea-urchin fertilized by radiated sperm has been made by G. Hertwig ('12). He found that after prolonged radiation the sperm retained their motility and entered the egg, but the sperm nucleus never increased to its normal size in the egg. As a rule it lay as a foreign body outside of the dividing egg nucleus, but occasionally it became involved in the spindle and the division was rendered abnormal, probably because of the mechanical interference. In such instances the sperm nucleus was carried passively into one of the blastomeres later fusing with the nucleus of that cell. The subsequent development of the cell and its descendants was abnormal. On the other hand, a shorter radiation injured the sperm nucleus but it fused with the egg nucleus. Later it was thrown out as in *Chætopterus*. In such instances the egg nucleus divided abnormally and cleavage

was irregular. Hertwig states that the abnormalities of the egg chromosomes are produced by the action of the injured sperm chromatin which operates like a poison. Its decomposition products in some way communicate the poison to the sound chromatin.

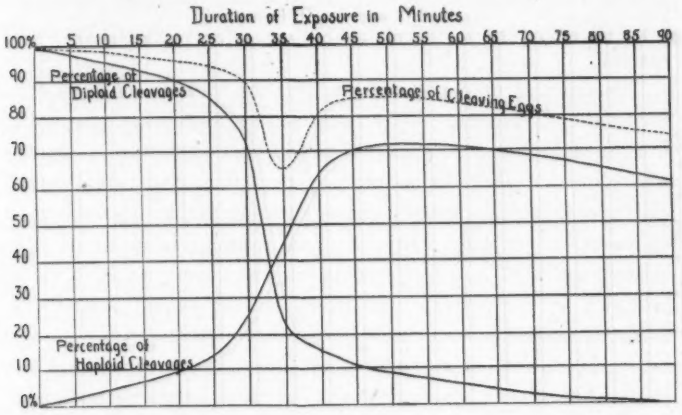
There is no evidence, other than that given by Hertwig, that radiated chromatin acts as a poison to the unradiated nucleus. His figures in support of this idea are not convincing; they can be explained more readily on the assumption that the injured chromatin acts merely as a mechanical hindrance in those instances in which it is actually caught in the cleavage spindle. It is difficult to imagine how disintegrating chromatin can communicate its injury to sound chromatin. The idea is apparently drawn from O. Hertwig's theory that the radiation produces a "contagium vivum" which acts in a manner similar to the poison elaborated by a pathogenic bacterium. Such an assumption has no basis in fact and does not explain the observed phenomena.

The cytological studies of Paula Hertwig on the eggs of *Triton* and the frog, and of Opperman on the trout, indicate that whatever effect the radiated chromatin produces in the division of the unradiated nucleus is due to mechanical interference. Such effects can best be seen when the radiated and sound nuclei actually fuse prior to the formation of the chromosomes. The radiated chromatin is eliminated from the spindle and causes a derangement in the spindle if it lies near enough to it. In *Chætopterus* the chromosomes form before the fusion of the nuclei and there is no evidence that any such interference occurs.

There is always a noticeable variation in the extent of injury produced by the radiations on a single lot of eggs. The curves shown in the text figure indicate that after the critical length of exposure, when the egg nucleus is greatly injured in the majority of cases, there are still some eggs which develop normally with the diploid number of chromosomes. This variation may be due to the fact that the eggs were not equally exposed, or that some are more resistant than others. The latter is probably the case. Paula Hertwig ('16) showed that when *Triton* eggs are exposed for a definite period some develop normally and some abnormally. The normal eggs in certain instances showed the

diploid number of chromosomes. Her explanation for this phenomenon is that the normal sperm chromosomes divided to form the diploid number at some time before the first cleavage. There is no evidence to prove this point. More probably the egg was not radiated sufficiently and the nucleus was comparatively uninjured so that it fused with the sperm nucleus and the subsequent cleavage was normal.

That sperms show varying resistance was shown by Gunther and Paula Hertwig ('13). When methylene blue is allowed to act



TEXT FIGURE 1.

for 18 hours on sperm from the same testis, some of the sperms are killed while others retain their motility perfectly. The same variation in susceptibility is probably found in the egg also.

Signs of injury in the chromatin do not appear until the chromatin increases in volume. The radiated sperm appears perfectly normal until it commences to grow inside of the egg. In the case of the sea-urchin it normally does not increase greatly in size until after it has fused with the egg nucleus, and it is not until that time that a radiated nucleus shows its abnormal condition. When the eggs of *Chætopterus* are radiated the chromosomes are in the form of tetrads, the two successive divisions of the chromatin having already taken place. The chromosomes divide regularly and do not increase noticeably in size. Polar body formation is

normal. It is abnormal only when the egg has been radiated so long that the protoplasm has been injured. But if the radiation is for periods up to 50 minutes, the polar bodies are well formed and the remaining chromosomes appear to be normal until they grow into the vesicular condition. The altered mode of development in such cases has already been described. If the radiation is short, that is, from 20 to 30 minutes, the chromatin does not show evidences of injury until the time of cleavage. Since chromatin grows by taking up substances from the protoplasm it is evident that the radiations have acted so as to interrupt this process, probably by altering the chemical constitution of the chromatin.

The unusual movement of the nucleus in radiated eggs calls for further comment. After short exposures the nucleus moves in a normal manner to a point slightly above the center of the egg where it fuses with the sperm nucleus. But as the duration of the exposure is lengthened it shows a greater and greater tendency to remain at the pole. In every case it makes some attempt to migrate inward, but its freedom of movement appears to be inhibited by the peculiar attachment to the second polar body. In many instances, especially in eggs radiated for 35 minutes or more, this curious attachment is not found, the nucleus lying free in the protoplasm. Following the extrusion of the second polar body, a process which is normal in all respects, the remaining chromosomes become vesicular and finally fuse to form a single large vesicle, which, instead of moving toward the sperm nucleus, remains in place. The chromatin which in the course of normal development condenses into chromosomes after the two nuclei have fused here develops into irregular masses, some of which may migrate slightly; the greater number do not. That chromatin which moves furthest from the pole is most normal in appearance.

Why does the egg nucleus fail to travel toward the center of the egg? The egg protoplasm has not been visibly injured; indeed it appears to be perfectly normal for it can draw in the sperm and the latter moves at the usual rate up to the center of the egg. In both monospermic and polyspermic eggs there is evidence that extensive protoplasmic movements are taking place for the yolk

spheres are pushed away from the advancing sperm so that the latter lies in an area of clear protoplasm. In respect to the sperm, therefore, the egg protoplasm reacts normally. Only the egg nucleus fails to migrate. As the duration of exposure increases the nucleus moves a shorter and shorter distance. In other words, the greater the injury to the chromatin, the smaller is the distance through which it moves. This is shown in Fig. 11. The egg nucleus under the polar bodies has formed into chromosomes and chromatin masses, of which the most abnormal in appearance is still in its original position while the rest have moved down to a position normally occupied by the egg nucleus at this stage. This condition suggests that the chromatin is concerned in its own movement.

This idea is supported by the behavior of the radiated chromatin shown in Figs. 8 and 9. In Fig. 8 the radiated chromatin in the form of chromosomes is left behind during the division of the sperm nucleus. One chromosome is normal in appearance but the spindle fiber attachment is abnormal; instead of being median it is terminal. In this instance there is an ineffectual attempt at movement. In Fig. 9 no spindle fibers are found attached to the irregular masses of egg chromatin, and the masses do not move with the sperm chromosomes.

According to Chambers ('17) the spindle fibers are lines of stress or of protoplasmic flow; they are not formed structures. As such they are probably produced as a result of the interaction of the chromosomes on the egg protoplasm. When the chromatin is rendered abnormal by the radiations it fails to stimulate their production and therefore does not move with the sperm chromosomes to which spindle fibers are attached. If the fibers develop under the influence of the chromosomes, there is no need to assume the presence of contractile fibers in the protoplasm which serve to pull the chromosomes toward the poles. They are not bodies which are passively pulled about in the cell, but are actively concerned in their own movement.

Summary.

1. Radiation of the unfertilized eggs of *Chætopterus* results in injurious effects which do not become manifest until after the

extrusion of the polar bodies, following insemination with normal sperm. That two normal mitotic divisions should intervene after radiation and only then that the injury to the chromosomes becomes apparent can be explained by the fact that the two longitudinal divisions preparatory to polar-body formation have been completed. Only when the chromosomes again "grow" in preparation for a new division do abnormal characters manifest themselves.

2. If the radiation is brief (20 to 30 min.) the germ nuclei fuse normally but the radiated egg chromatin fails to form normal chromosomes. No spindle fibers develop from these abnormal chromatin masses, and they remain in the cleavage plane during mitosis. If the radiation is longer (35 to 50 min.) the chromosomes, after the extrusion of the polar bodies, develop abnormally and fail to fuse with the sperm nucleus. In both cases the sperm nucleus divides and development is androgenetic.

3. During cleavage the most injured chromatin does not move and lacks spindle fibers. This suggests that the fibers are normally developed under the influence of the chromosomes and that the latter are concerned in their own movement.

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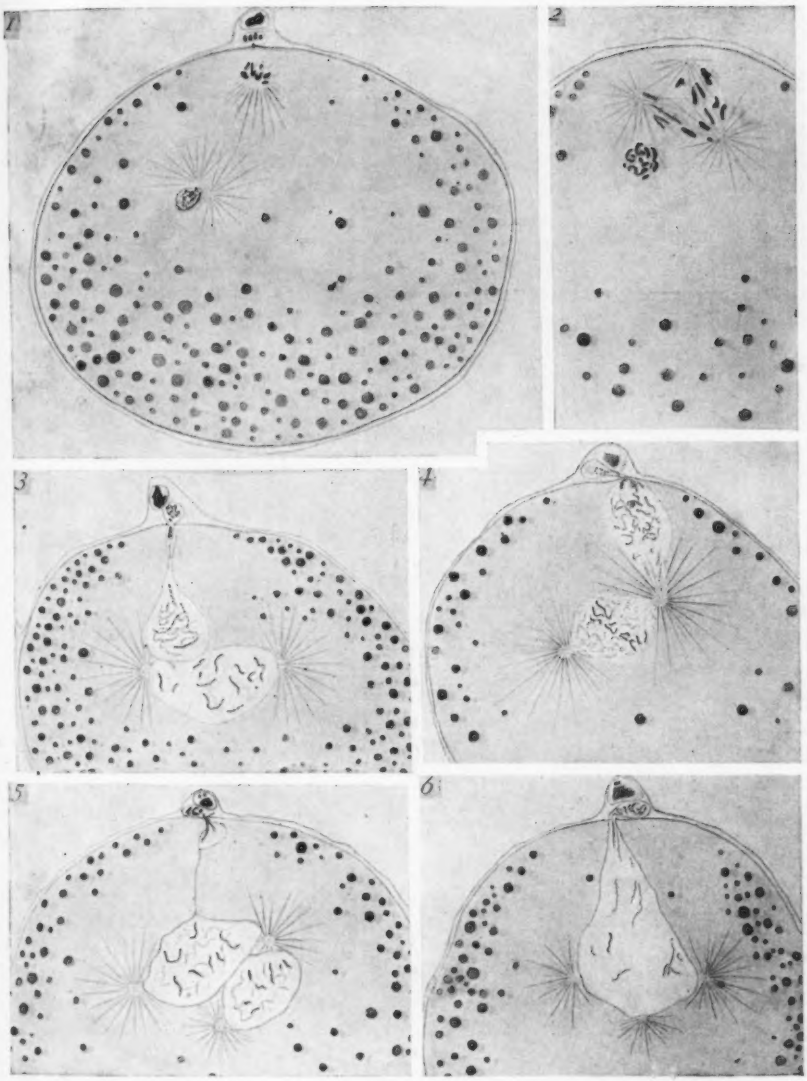
EXPLANATION OF PLATES.

The drawings were made with the aid of a camera lucida. The lenses employed were a 1.5 mm. Zeiss Apochromatic and a No. 6 Compensating ocular.

Explanation of Plate I.

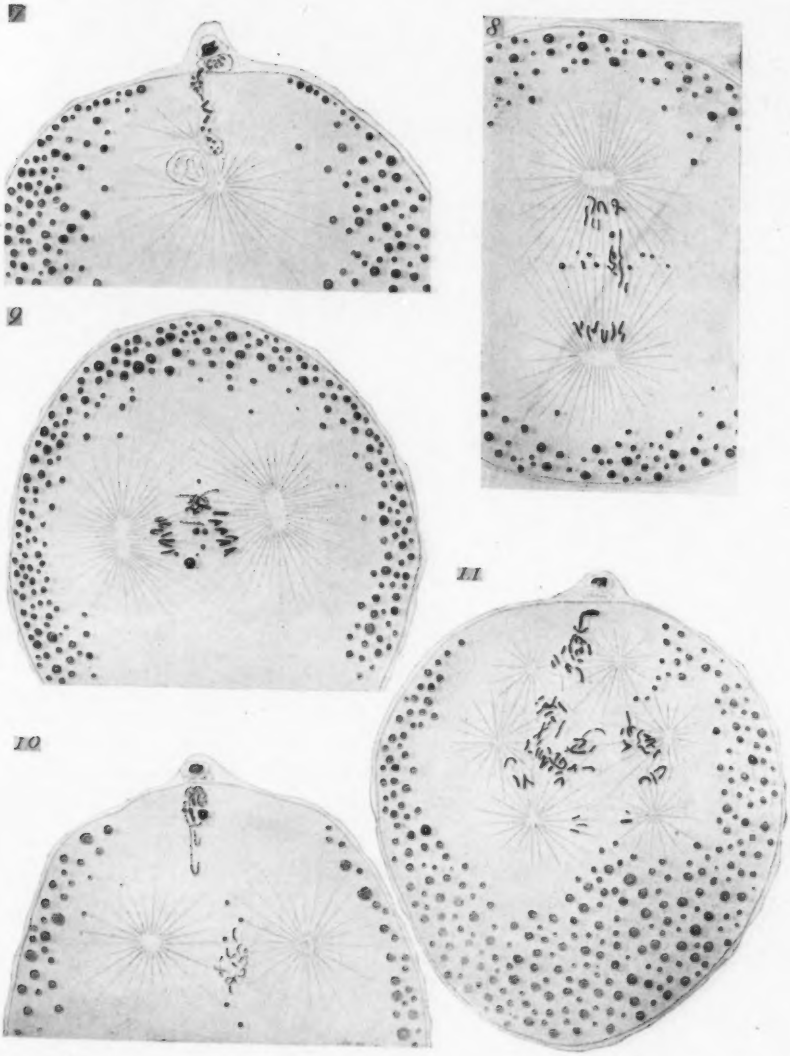
Fig. 1. Normal fertilization. 25 min. after insemination.
Fig. 2. Egg radiated for 80 min. The development of the sperm is abnormal.
Fig. 3. Egg radiated 35 min. Killed 34 min. after insemination.
Fig. 4. " " " " " 30 min. " "
Fig. 5. " " " " " 39 min. " "
Fig. 6. " " " " " 39 min. " "



CHARLES PACKARD.

Explanation of Plate II.

Fig. 7.	Egg radiated 35 min.	Killed 34 min. after insemination.	
Fig. 8.	Egg radiated 25 min.	" 42 min. "	"
Fig. 9.	Egg radiated 35 min.	" 39 min. "	"
Fig. 10.	" " " "	" 34 min. "	"
Fig. 11.	" " " "	" 40 min. "	"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